Universidade de Brasília
Instituto de Ciências Exatas
Departamento de Ciência da Computação

Uma extensão do sistema de anotação de RNAs não-codificadores ncRNA-Agents para plantas: um estudo de caso para o Zea mays

Raquel Ingrid da Silva Nunes

Monografia apresentada como requisito parcial
para conclusão do Curso de Engenharia da Computação

Orientadora
Prof.ª Dr.ª Maria Emília Machado Telles Walter

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Dedicatória

Dedico este trabalho a minha família, meus pais e meus irmãos, que fizeram o impossível para que eu pudesse chegar até aqui. Vocês são a minha base, obrigado por tudo.

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Resumo

RNAs não-codificadores (*non-coding RNAs*-ncRNAs) participam de uma gama notável de reações biológicas e processos celulares, e diversos métodos e algoritmos vem sendo desenvolvidos para identificá-los e classificá-los. Em particular, o ncRNA-Agents é um sistema de anotação de ncRNAs baseado no conceito de Sistemas Multiagentes (SMAs), criado inicialmente para anotação de fungos. Neste trabalho, foi criada uma nova instância do ncRNA-Agents para anotação de ncRNAs em plantas. Foi realizado um estudo de caso para a espécie *Zea mays* (milho), com o objetivo de predizer ncRNAs pequenos em transcritos de oito bibliotecas, sendo quatro inoculadas com as bactérias *Azospirillum* e *Herbaspirillum* e quatro foram de controle. Foi inicialmente proposto um *pipeline* com as seguintes etapas: (i) os transcritos de todas as bibliotecas foram mapeadas no genoma do *Z. mays*, tendo sido obtidas as sequências consenso. (ii) Dessas foram removidas as sequências de proteínas e aquelas com mais de 200 *bp*. As sequências com menos de 200 *bp* foram submetidas ao ncRNA-Agents, para anotação de ncRNAs pequenos. Aproximadamente 18.300 sequências foram submetidas como entrada, tendo sido encontrados diferentes tipos de ncRNAs, em sua maioria RNAs transportadores (tRNAs) e *micro* RNAs (miRNAs), sendo encontrados também RNAs ribossomais (rRNA), *Small nucleolar* RNAs U3 (snoRNA U3), *Plant signal recognition particle* RNA (Plant SRP) e U2 *spliceosomal* RNA (snRNA U2).

Palavras-chave: RNA, RNAs não-codificadores pequenos, ncRNA-Agents, anotações de RNAs não-codificadores pequenos, *Zea mays*.

Abstract

Non-coding RNAs (ncRNAs) participate in a remarkable range of biological reactions and cellular processes, and a number of methods and algorithms have been developed to identify and classify them. In particular, ncRNA-Agents is a ncRNAs annotation system based on the concept of Multiagent Systems (SMAs), initially created for fungal annotation. In this work, we created a new instance of ncRNA-Agents for annotation of ncRNAs in plants. A case study for *Z. mays* (maize) was carried out to predict small ncRNAs in transcripts from eight libraries, four of which were inoculated with the*Azospirillum* and *Herbaspirillum* bacteria, and four were of control. A pipeline was initially proposed with the following steps: (i) the transcripts of all the libraries were mapped into the *Z. mays* genome, and the consensus sequences were obtained. (ii) Of these, protein sequences and those with more than 200 bp were removed. Sequences with less than 200 bp were submitted to the ncRNA-Agents, for annotation of small ncRNAs. Approximately 18,300 sequences were submitted as input, and different types of ncRNAs were found, mostly transporter RNAs (tRNAs) and microRNAs (miRNAs), besides ribosomal RNAs (rRNA), Small Nucleolar RNAs U3 (snoRNA U3), Plant Signal Recognition Particle RNA (Plant SRP) and U2 spliceosomal RNA (U2 snRNA).

Keywords: RNA, small non-coding RNAs,ncRNA-Agents, annotations of small non-coding RNAs, *Zea Mays*.

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Capítulo 1

Introdução

A ciência moderna mostra que a vida surgiu na Terra aproximadamente há 3.5 bilhões de anos [50]. As primeiras formas de vida eram muito simples, mas com o passar dos anos houve um grande processo de evolução que resultou na vida como ela é conhecida atualmente.

Todas as formas de vida têm uma química molecular parecida. Os principais componentes dessa química são as proteínas e os ácidos nucleicos. Proteínas são responsáveis por aquilo que um ser vivo é e faz em um sentido físico, enquanto os ácidos nucleicos codificam a informação necessária para produzir proteínas e são responsáveis por passar a "receita" para as gerações seguintes [50], ou seja, são responsáveis pelo armazenamento e transmissão da informação genética. O ácido desoxirribonucléico (DNA) e ácido ribonucléico (RNA) são tipos de ácidos nucleicos.

Depois dos estudos de Watson e Crick em 1953 [65], onde foi proposta uma estrutura de dupla hélice para a molécula do DNA, a comunidade científica vem fazendo grande esforço para tentar compreender melhor a estrutura e o funcionamento da biologia molecular dos organismos. A área de Biologia Molecular, por sua vez, é voltada basicamente para o entendimento das estruturas e as funções das proteínas e dos ácidos nucleicos.

Nas décadas de 1950 e 1960, não era nada simples obter uma sequência de DNA, fosse ela fita simples ou dupla. De fato, o conhecimento sobre os ácidos nucleicos avançava de forma lenta. Porém no início da década de 80, uma técnica relativamente rápida de sequenciamento de DNA foi desenvolvida, que empregava a quebra de uma cadeia de DNA com diferentes produtos químicos e, em seguida, apresentava uma visualização dos fragmentos gerados por eletroforese. Havia necessidade de fazer a marcação radioativa das moléculas porque a quantidade de material produzida era muito pequena e não havia outra forma de obter essas moléculas. Mesmo com todas estas dificuldades, houve então um rápido progresso no conhecimento de sequências de DNA [61].

O Projeto Genoma Humano [64] [32] teve início em 1990, e foi um consórcio internacional que objetivou o mapeamento completo de todos os cromossomos e sequências de DNA de humanos. O projeto recebeu um financiamento de 3 milhões de dólares do Departamento de Energia dos Estados Unidos e do Instituto Nacional de Saúde dos Estados Unidos. O projeto foi concluido em 2001 e sequenciou 3 bilhões de bases, ou seja, teve um sequenciamento de 99% do genoma humano com uma precisão de 99,99 % [31].

Até o início da década de 90, as sequências de DNA eram obtidas com técnica manual, o que certamente demandou muito tempo e trabalho. Ainda na década de 70, foi proposto

por Frederick Sanger o método Sanger [49], que consistia em adicionar nucleotídeos modificados, os didesoxiribonucleotídeos. Estes impedem o crescimento de um fragmento de DNA em replicação pela DNA polimerase após sua adição. A técnica ainda é utilizada nos dias atuais, embora existam hoje em dia sequenciadores automáticos que proporcionam cada vez mais precisão na obtenção de porções de DNA e RNA.

Devido aos avanços tecnológicos e ao enorme volume de dados gerados em larga escala, as ferramentas precisam de cada vez mais poder computacional, para que as análises possam ser realizadas mais rápida e precisamente. Para trabalhar com os dados dentro da Biologia Molecular, métodos e técnicas de Matemática, Estatística e Ciência da Computação são normalmente utilizados. Neste contexto, surgiu a **Bioinformática**, uma área do conhecimento que utiliza ferramentas e técnicas computacionais para interpretar e analisar informações geradas pelos sequenciadores automáticos [37]. Nos projetos dessa área, a complexidade dos problemas e o grande volume de dados, requerem técnicas sofisticadas de computação.

A comparação das sequências é a operação primitiva mais importante na Bioinformática, servindo como base para outras análises mais complexas. Esta operação consiste em constatar que partes das sequências são similares e que partes são diferentes. No entanto, por trás desse conceito aparentemente simples, há uma grande variedade problemas distintos, com diversas formalizações e, muitas vezes, exigindo estruturas de dados e algoritmos complexos e eficientes.

Voltando um pouco no tempo devemos destacar um fator importante para o avanço da Biologia Molecular: o aprofundamento do trabalho de Watson e Crick (1953), em que Crick (1958), propôs o Dogma Central da Biologia Molecular [13], que revela que, através da transcrição, determinadas áreas da molécula de DNA, transformam-se em mRNA (RNA mensageiro) e este por sua vez é sintetizado em proteína através dos RNAs ribossomal (rRNA) e transportador (tRNA), pelo processo conhecido como tradução.

O conhecimento sobre as moléculas de ácidos ribonucleicos, os RNAs, estava basicamente ligado ao uso da informação contida no DNA para tradução de proteínas. Porém, com o tempo foi descoberto outro tipo de molécula de RNA, não traduzida em proteína, hoje denominada de RNA não-codificador de proteína (ncRNA). Antigamente considerados como RNA lixo, os ncRNAs não eram considerados para a análise do genoma. A partir do século XXI, o ncRNA começou a ser mais profundamente pesquisado, devido a sua característica de, mesmo sem ser traduzido em proteína, ter papéis importantes nos mecanismos celulares, como regulação.

Os ncRNAs participam de uma gama notável de reações biológicas e processos, como a iniciação de tradução, controle de abundância de mRNA, arquitetura do cromossomo, manutenção de células-tronco, desenvolvimento do cérebro, músculos e e secreção de insulina, dentre outras [40].

Apesar de tantas funções importantes, os ncRNAs ainda não podiam ser identificados e classificados até pouco tempo atrás. Além disso, do ponto de vista computacional, o fato de sequências de certas classes de ncRNAs serem curtas e não terem um padrão de sequência bem comportado impede que eles sejam reconhecidos apenas por suas bases. Dessa forma, eles devem ser identificados por suas estruturas secundárias (espaciais).

O sistema de anotação de ncRNAs, chamado de ncRNA-Agents, é baseado no conceito de Sistemas Multiagentes (SMAs), que dentro da Inteligência Artificial, são caracterizados pela distribuição de inteligência entre diferentes entidades autônomas (agentes), que inte-

ragem para atingir objetivos individuais e coletivos. Para tanto, os agentes que compõem um SMA podem negociar, cooperar para atingir objetivos (que não podem ser realizados por um único agente) e coordenar esforços conjuntos [67].

Utilizar resultados de vários programas em um SMA finalmente possibilitou anotações mais confiáveis de ncRNAs. A proposta deste trabalho é fazer uma extensão do ncRNA-Agents, para a análise de ncRNAs de plantas.

1.1 Motivação

No século XXI, a identificação, a classificação e a anotação de ncRNAs constituem-se em pesquisas desafiadoras, tanto em Biologia Molecular quanto em Bioinformática, devido às descobertas recentes de que esses ncRNAs exercem diferentes funções e papéis importantes nos mecanismos celulares [1]. Assim, existe a necessidade de um estudo mais aprofundado dessas moléculas. Ainda hoje são grandes os desafios para identificar, anotar e classificar ncRNAs. Neste contexto, o ncRNA-Agents tem o objetivo de anotar ncRNAs, baseando-se em sistema multiagente (SMA) e simulando o raciocíno dos biólogos por regras declarativas [3]. Por outro lado, há necessidade de estudo focado em plantas, pois não existem muitos estudos sobre os ncRNAs em plantas.

1.2 Problema

Não há análises de ncRNAs do *Zea mays* (milho). Foram sequenciadas na UFRJ, oito bibliotecas do *Z. mays*, quatro tratadas com bactérias diazotróficas, e quatro funcionando como controle. Em particular, tanto quanto é conhecido, não há anotação de ncRNAs pequenos do milho.

1.3 Objetivos

O objetivo principal deste trabalho é criar uma nova instância do ncRNA-Agents para plantas das famílias *Gramíneas* e *Solanaceae*.

Os objetivos específicos são:

1. Propor uma nova instância do ncRNA-Agents, incluindo bancos de dados de plantas, e modificando as regras do raciocínio do sistema de forma adequada;
2. Realizar um estudo de caso para ncRNAs pequenos do *Z. mays*;
3. Relatar os resultados obtidos do estudo de caso.

1.4 Descrição dos Capítulos

No Capítulo 2 serão apresentados os conceitos básicos de Biologia Molecular e, em seguida, serão explorados os conceitos de ncRNAs.

No Capítulo 3 serão apresentadas definições de agentes e sistemas multiagentes, além da descrição do sistema atual do ncRNA-Agents.

No Capítulo 4 será descrita a nova instância do ncRNA-Agents, com aspectos importantes da implementação, sendo monitorados os novos bancos de dados incluídos. Também neste serão discutidos os resultados do estudo de caso feitos para o milho.

Por fim, no Capítulo 5 será concluído este trabalho e serão propostos os trabalhos futuros.

Capítulo 2

Biologia Molecular

Neste capítulo serão discutidos conceitos de Biologia Molecular [4] necessários para o entendimento deste projeto. Na Seção 2.1, detalhamos ácidos nucleicos e proteínas, já na Seção 2.2 será detalhado o Dogma Central da Biologia Molecular (síntese de proteínas). Na Seção 2.3 serão discutidos os conceitos relacionados a RNAs não-codificadores.

2.1 Ácidos Nucleicos

Ácidos nucleicos [65] são macromoléculas compostas por unidades menores, conhecidas por nucleotídeos, ou seja, são cadeias de nucleotídeos. Eles são:

1. Uma molécula de açúcar (pentose) (Figura 2.1);
2. Um grupamento fosfórico (fosfato);
3. Uma base nitrogenada: Adenina, Guanina, Citosina e Timina (DNA)/Uracila (RNA).

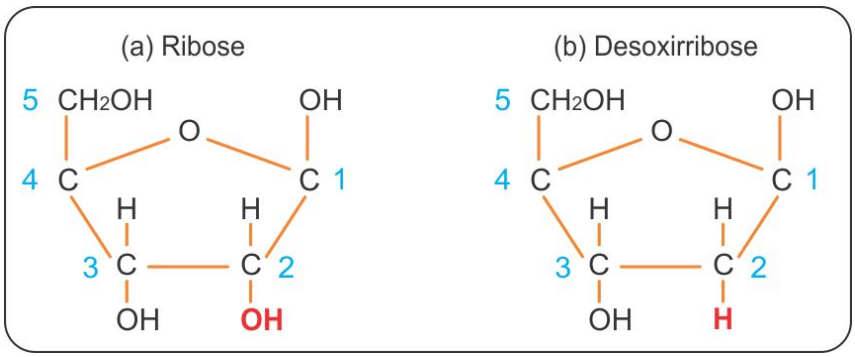


Figura 2.1: Representação dos dois tipos de pentoses. (a) A pentose do RNA possui o grupo hidroxila (OH) no carbono 2; (b) A pentose de DNA possui um átomo de hidrogênio (H) ligado ao carbono 2 [24].

Os organismos vivos contêm dois tipos de ácidos nucleicos: ácido ribonucleico, abreviado como RNA, e ácido desoxirribonucleico ou DNA [50].

2.1.1 DNA

No DNA estão contidas as informações genéticas dos seres vivos. Nos organismos eucariotos (cujas células contêm núcleos), o DNA está localizado no núcleo. Como dito antes, a molécula do DNA é formada por uma molécula de açúcar 2'-deoxiribose ligada a um resíduo de fosfato [50]. A pentose (áçucar simples) tem cinco átomos de carbono numerados de 1' a 5'. Através dos grupos fosfatos, é feita a ligação de nucleotídeos para formar uma fita de DNA, possibilitando assim a ligação do carbono 3' de um nucleotídeo a um grupo fosfato, que se liga a outro nucleotídeo através do carbono 5'. Por esse motivo, há a convenção de que os ácidos nucleicos são formados numa direção 5'→ 3', adotada como direção canônica.

A estrutura do DNA é formada por duas cadeias ou fitas paralelas (Figura 2.2) compostas por uma sequência de nucleotídeos, que são unidas através de pontes de hidrogênio criadas entre as bases nitrogenadas de cada fita. As duas fitas ficam unidas pela junção das bases complementares A/T (Adenina/Timina), C/G (Citosina/Guanina).

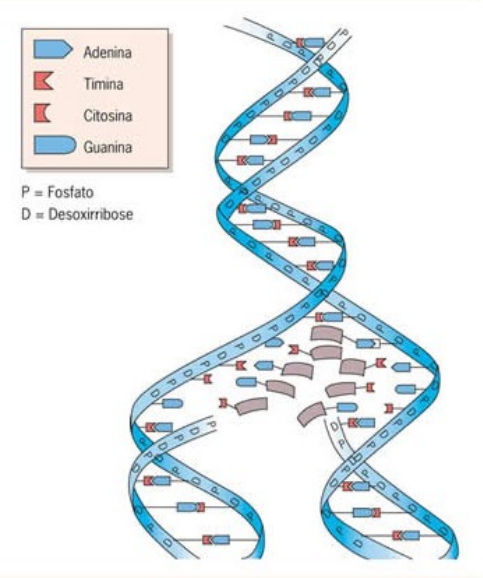


Figura 2.2: Esquema de duplicação do DNA [17].

Observa-se também que o DNA pode sofrer um processo de replicação (Figura 2.2), em que uma molécula de DNA é capaz de formar outra molécula idêntica à original. Neste processo, as pontes de hidrogênio existentes entre as bases se rompem e as duas cadeias começam a se separar. Enquanto isso acontece, nucleotídeos disponíveis no meio vão se unindo às bases, sempre respeitando a complementaridade: A com T, T com A, C com G e G com C. Uma vez ordenados sobre a cadeia que está servindo de modelo, os nucleotídeos ligam-se em sequência e formam uma cadeia complementar sobre cada uma das cadeias da molécula original. Hoje também se sabe que uma molécula de RNA pode produzir DNA. Chamamos esse processo de transcrição reversa e ele acontece principalmente em retrovírus.

O material genético contido no DNA precisa ser traduzido em proteínas, que atuarão nas reações metabólicas da célula. Assim as informações contidas no DNA devem ser passadas para a molécula de RNA, que posteriormente irá atuar na síntese de proteínas.

O controle da atividade celular pelo DNA é feito indiretamente e ocorre com a fabricação de moléculas de RNA, é o conhecido como processo de transcrição (Figura 2.3).

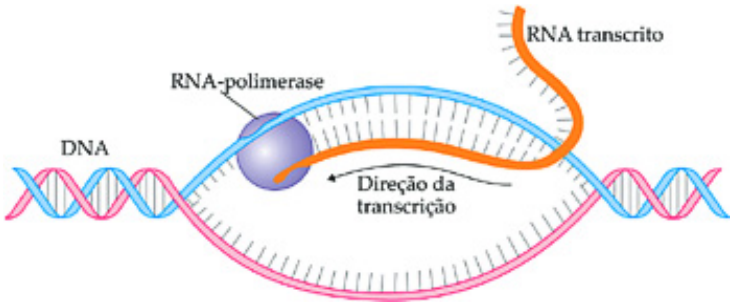


Figura 2.3: Processo de transcrição [60].

2.1.2 RNA

Existem três diferenças principais entre o DNA e o RNA. No RNA estão presentes:

1. A ribose no lugar da desoxirribose;
2. A presença da Uracila no lugar da Timina;
3. Apenas uma cadeia de nucleotídeos, em geral.

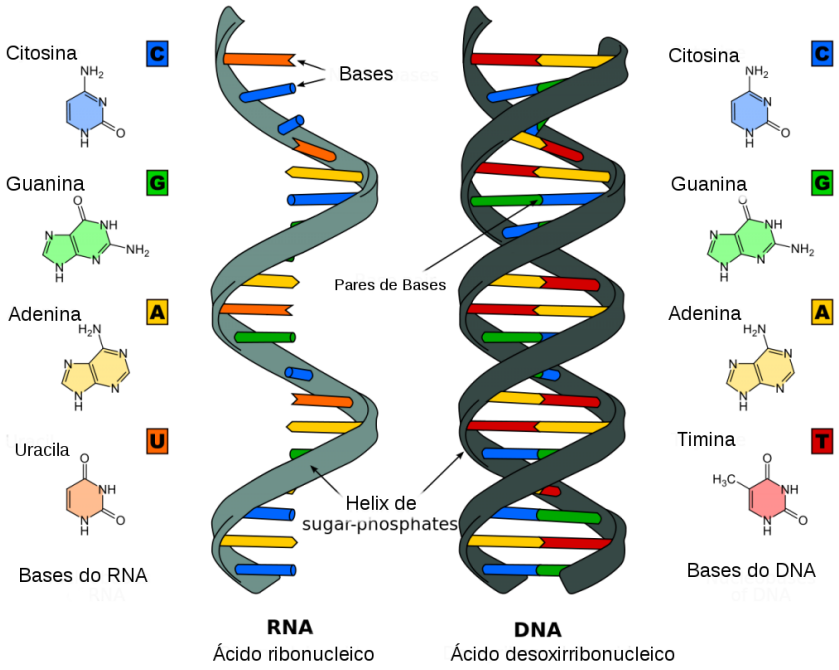


Figura 2.4: Esquemas do DNA e do RNA (adaptado de [51])

As moléculas de RNA envolvidas na síntese de proteínas, detalhadas a seguir, são:

- *RNA ribossômico*, que está relacionado à produção de proteínas na célula;

- *RNA mensageiro*, que leva as informações da DNA para o RNA ribossômico;
- *RNA transportador*, que é o responsável por transportar os aminoácidos que serão utilizados na síntese de proteínas.

A Figura 2.4 mostra um esquema do DNA e do RNA.

2.1.3 Proteínas

No processo de tradução, ocorre a formação dos aminoácidos. A informação contida no DNA é formada pelos quatro nucleotídeos: A, T, C e G. Fazendo uma pequena analogia com a língua portuguesa: A, T, C e G são "letras", com essas quatro letras é preciso formar "palavras", que são os aminoácidos, e cada proteína corresponde a uma "frase" formada pelas "palavras" (aminoácidos).

Vinte aminoácidos estão presentes nos seres vivos, sendo cada aminoácido formado por três letras (uma trinca de bases) do DNA. Cada trinca de bases, é denominada **códon** e cada códon corresponde a um certo aminoácido. A Tabela 2.1 mostra a correspondência entre os códons e os aminoácidos. No entanto, um mesmo aminoácido pode ser definido por mais de um códon. Os códons UAG, UGA e UAA determinam o final da tradução, sendo representado, na Tabela 2.1, como Fim (em inglês *stop códon*).

Tabela 2.1: Correspondência entre códons e aminoácidos [68].

Primeira base	Segunda base U	Segunda base C	Segunda base A	Segunda base G	Terceira base
U	UUU Fen UUC UUA Leu UUG	UCU UCC UCA Ser UCG	UAU Tir UAC UAA Fim UAG	UGU Cis UGC UGA Fim UGG Trp	U C A G
C	CUU CUC CUA Leu CUG	CCU CCC CCA Pro CCG	CAU His CAC CAA Gln CAG	CGU CGC CGA Arg CGG	U C A G
A	AUU AUC AUA Ile AUG Met	ACU ACC ACA Tre ACG	AAU Ans AAC AAA Lis AAG	AGU Ser AGC AGA Arg AGG	U C A G
G	GUU GUC GUA Val GUG	GCU GCC GCA Ala GCG	GAU Asp GAC GAA Glu GAG	GGU GGC GGA Gli GGG	U C A G

A Tabela 2.2 traz uma lista dos aminoácidos mais comumente encontrados na natureza.

2.2 Dogma Central da Biologia Molecular

O *Dogma Central da Biologia Molecular*, proposto por Francis Crick em 1958 [13], explica como ocorre o fluxo de informações do código genético em organismos. Esse

Tabela 2.2: Os vinte aminoácidos mais comumente encontrados na natureza e suas estruturas químicas correspondentes [50].

Abreviatura	**Nome**
Ala	Alanina
Cys	Cisteína
Asp	Aspartato
Glu	Glutamato
Phe	Fenilalanina
Gly	Glicina
His	Histidina
Ile	Isoleucina
Lys	Lisina
Leu	Leucina
Met	Metionina
Asn	Asparagina
Pro	Prolina
Gin	Glutamina
Arg	Arginina
Ser	Serina
Thr	Treonina
Val	Valina
Trp	Triptofano
Tyr	Tirosina

modelo mostra que uma sequência de DNA pode formar uma proteína, com auxílio de diferentes RNAs. Segundo esse dogma, o fluxo da informação genética segue no sentido DNA→proteína (Figura 2.5).

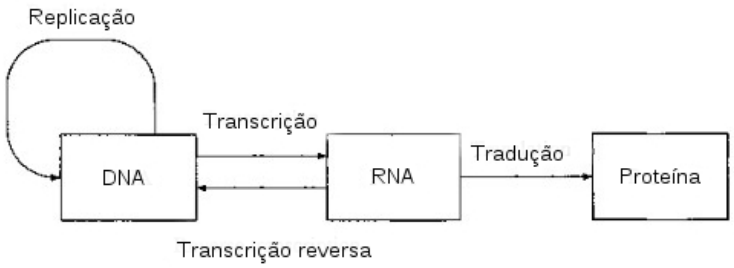


Figura 2.5: Esquema do Dogma Central da Biologia Molecular [50].

Porém, algumas mudanças já foram propostas no modelo original. Isso ocorreu em razão de hoje se saber, por exemplo, que nem todos os RNAs produzem proteínas.

O Dogma Central da Biologia Molecular pode ser resumido da seguinte maneira: a formação de uma proteína começa no DNA, onde está contida a informação genética, que pode ser transcrita em moléculas de RNA. Na transcrição, um pedaço da molécula

de DNA serve como molde para a criação de uma molécula de RNA (RNA mensageiro - mRNA), lembrando que caso o organismo seja procarioto o mRNA já estará pronto para sintetizar uma proteína. Em um organismo eucarioto, antes da formação de um mRNA maduro ocorre o processo de *splicing*. Quando o mRNA é formado, a partir do DNA, regiões conhecidas como *éxons*[1] são alternadas com regiões conhecidas como *íntrons*[2] que contém informações aparentemente não utilizadas. Assim, para que a proteína seja criada, o pré-m*RNA* é copiado do DNA, essa cópia então passa pelo processo de *splicing*, no qual os *íntrons* são removidos, restando apenas o mRNA maduro formado apenas de *éxons*. A Figura 2.6 mostra o processo de formação do mRNA.

Depois de formado, o mRNA maduro guia a produção de um aminoácido usando o RNA transportador (tRNA) que, em uma ponta possui três bases (*codón*), e na outra ponta o aminoácido correspondente. A sequência de aminoácidos (Tabela 2.1) produzida pelos tRNAs formam as proteínas. O processo de geração de aminoácidos - tradução, a partir de um *codón*, ocorre no ribossomo, uma estrutura formada por RNAs ribossômicos (rRNA).

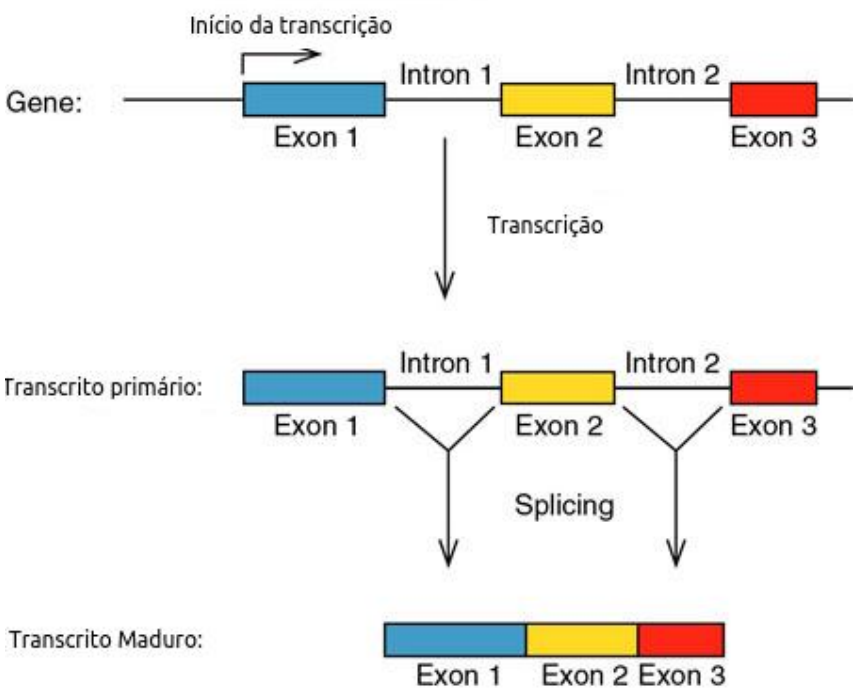


Figura 2.6: Processo de *Splicing*.

O DNA complementar (cDNA) é o DNA sintetizado a partir de uma molécula de mRNA, que já sofreu o processo de *splicing*.

2.3 RNAs não-codificadores

Nesta seção, descrevemos RNAs não-codificadores (*non-coding RNA - ncRNAs*) com mais detalhes.

[1]Trecho contíguo de uma sequência de DNA que vai ser utilizado para construir o mRNA maduro.
[2]Trecho contíguo do DNA que é descartado no processo de *splicing*.

2.3.1 Descrição

NcRNAs são RNAs funcionais que não são traduzidos para proteínas. Um sinônimo menos utilizado é RNA não-codificador de proteínas.

Na década de 80, os ncRNAs eram considerados como RNAs lixo (*junk* RNA), sendo desconsiderados para análise do genoma. Apenas a partir do século XXI, o ncRNA começou a ter um espaço maior nas pesquisas, devido a sua característica de, mesmo sem ser traduzido em proteína, participar de diversas funções nos mecanismos celulares. Estudos já revelaram que cerca de 98% do que é transcrito pelo genoma humano é constituído de ncRNAs [63].

A sequência do DNA da qual é transcrito um RNA não codificador é denominada gene não codificador de proteína.

Como pode ser observado na Figura 2.7, as funções reguladoras de ncRNAs estão envolvidas em todas as etapas do Dogma Central da Biologia Molecular, na transcrição, pós-transcrição e tradução. A informação genética flui do DNA (genótipo) para o RNA (com linhas pretas e grossas). O RNA (fenótipo) codifica proteínas e/ou ncRNAs a partir de mRNAs. Os ncRNAs controlam a regulação da expressão gênica (como podemos ver nas linhas tracejadas), ao passo que ncRNAs estruturais (por exemplo, rRNA e tRNA) estão envolvidos na síntese de proteínas [3].

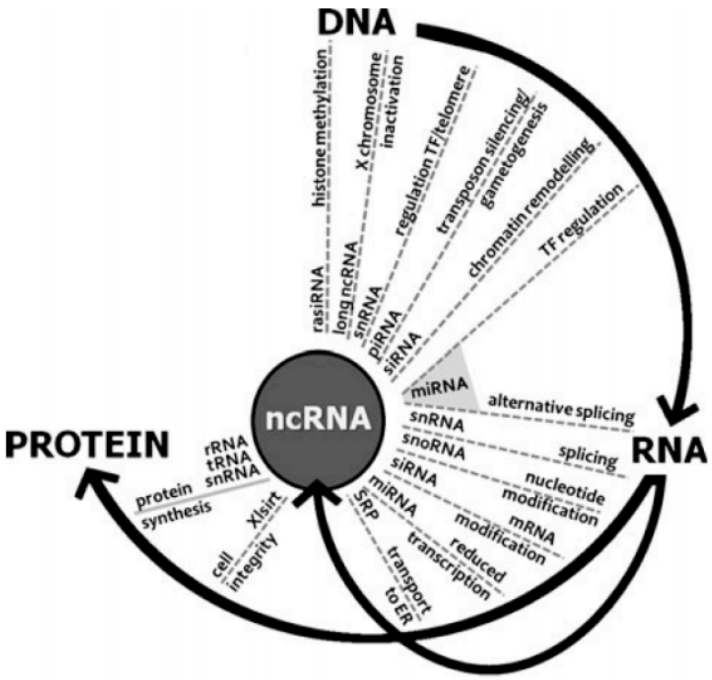


Figura 2.7: NcRNAs ligados ao Dogma Central da Biologia. As linhas contínuas indicam o fluxo de informação - do DNA para proteína - as linhas pontilhadas associam a cada classe de ncRNA seu papel biológico [36].

2.3.2 Classes

Classes de ncRNAs podem ser distinguidas por suas funções, que dependem diretamente da estrutura e comprimento das suas moléculas, e da composição de suas sequências. Os ncRNAs tem pequenas estruturas chamadas estruturas secundárias (serão definidas mais à frente) que tendem a dobrar-se de diferentes formas, sendo que essas estruturas conferem funcionalidades a esses RNAs.

A classificação de ncRNAs é feita, em geral, de acordo com suas funcionalidades, o que permite entender o papel que os ncRNAs realizam nos mecanismos celulares. O número de famílias atualmente conhecidas já ultrapassa 2.450 [1], embora mesmo quando uma função seja conhecida, os mecanismos moleculares subjacentes sejam ainda mal compreendidos.

Nos genes de RNA não-codificadores pequenos (20 - 30 nucleotídeos) incluem-se os RNAs de transferência (tRNAs), os RNAs ribossômicos (rRNAs) e os pequenos (*small*) RNAs, tais como os snoRNA, microRNA, siRNA e piRNA (Figura 2.8). Aqui encontram-se as definições dos principais tipos de RNAs pequenos [3]:

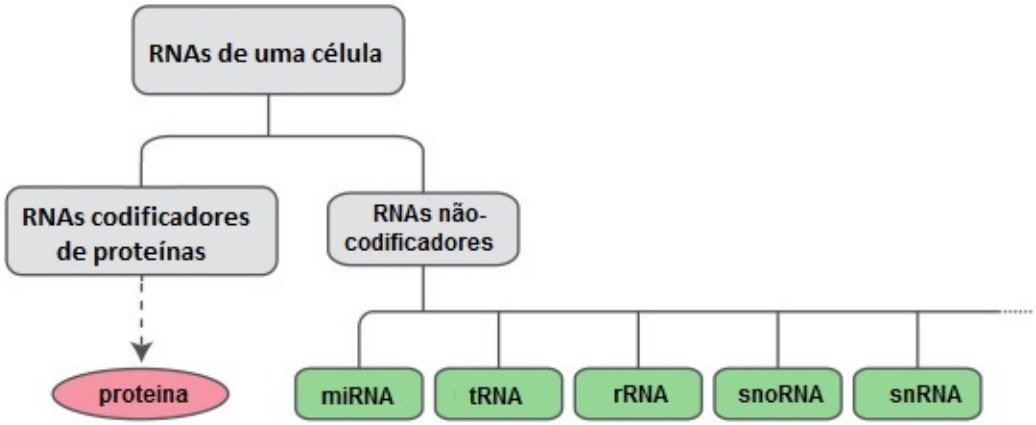


Figura 2.8: Classes de RNAs [8].

1. RNA transportador (tRNA): é utilizado como molécula transportadora de informação de cada códon componente do mRNA em um aminoácido específico, a ser adicionado à proteína sendo formada (Figura 2.9). Desempenha sua função através de duas regiões, o anticódon, que é responsável pelo reconhecimento de códons específicos do mRNA, e o aminoácido correspondente ao códon;

2. RNA ribossomal (rRNA): componente central do ribossomo, sua função é providenciar um mecanismo para decodificar o mRNA em aminoácidos e interagir com os tRNAs durante a tradução;

3. *Small nuclear RNA* (snRNA): encontrado no núcleo da célula, envolve-se no processo de *splicing* do pré-mRNA, em que os íntrons de um transcrito primário são eliminados, levando a um mRNA maduro;

4. *Small nucleolar RNA* (snoRNA): classe de pequenas moléculas que fazem modificações químicas no rRNA, e em outros ncRNAs, como o tRNA, com o objetivo de promover a maturação desses ncRNAs, transformando-os em moléculas ativas;

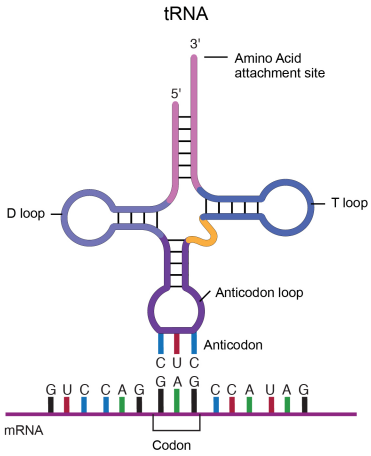


Figura 2.9: Estrutura do tRNA [29].

5. *Small Cajal body-specific RNA* (scaRNA): classe de snoRNAs que estão localizadas especificamente no *Cajal body*[3]. Há evidências de scaRNAs compostos por snoRNAs tanto H/ACA box quanto C/D box, por exemplo, snoRNA U85;
6. MicroRNA (miRNA): são moléculas complementares a uma ou mais moléculas de mRNA, sua função principal é silenciamento pós-transcricional, pareando-se com mRNAs para regular sua tradução e estabilidade;
7. *Small interfering RNA* (siRNA): reduz a expressão de genes codificadores, degradando o mRNA maduro;
8. *Piwi-interacting RNA* (piRNA): pequenas moléculas de RNA existentes nas células dos mamíferos, também estão relacionados com a regulação gênica;
9. *Small non-messenger RNAs* (snmRNAs): possuem a função de regulação.

A Tabela 2.3 resume as funções descritas acima.

2.3.3 Estruturas

Nos ncRNAs os três componentes estruturais são talo, alça e grampo:

- Talo *(stem)*: contém pares de bases complementares [3];
- Laço*(loop)*: local de não pareamento das bases [3];
- Grampo*(hairpin)*: um *loop* encerrado por uma hélice [3].

A estrutura do ncRNA pode ser observada na Figura 2.10.

Foram criadas várias abstrações para facilitar o entendimento das estruturas das moléculas de ncRNAs. Os três mais usados estão descritos a seguir (Figura 2.11):

[3]Os *Cajal bodies* (CBs), também conhecidos como *coiled bodies*, são suborganelas esféricas encontradas no núcleo de células proliferativas, como células embrionárias e células tumorais, ou células metabolicamente ativas, como neurônios.

Tabela 2.3: Alguns tipos de ncRNAs e suas funções conhecidas (adaptado de [3]).

Sigla	**Funções**
RNA transportador [43]	Transporta informação de códon componente do mRNA em um aminoácido específico
rRNA [20]	RNA constituinte do ribossomo
snRNAs [5, 33, 62]	Envolvido no processo de *splicing*
snoRNAs [19]	Envolvido na modificação do rRNA
miRNA [39]	Família putativa de genes reguladores da tradução
siRNA [20]	Ativas na interferência de RNA
piRNA [7]	Regulação de tradução e estabilidade de mRNA, entre outras
scaRNA [14, 16]	Função similar a dos snoRNAs

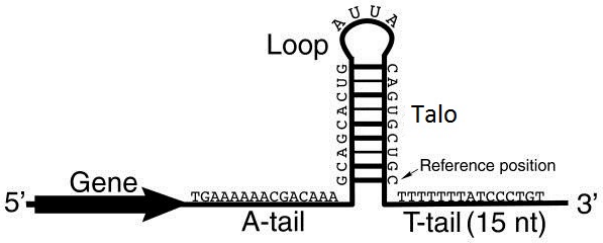


Figura 2.10: Estrutura do ncRNA [59].

- Estrutura primária: a sequência de bases que define a molécula. Essa sequência pode ser gerada pelos sequenciadores automáticos;
- Estrutura secundária: representação da estrutura 2D de um RNA, mostrando as ligações entre os pares de bases complementares;
- Estrutura terciária: representação espacial 3D de um RNA.

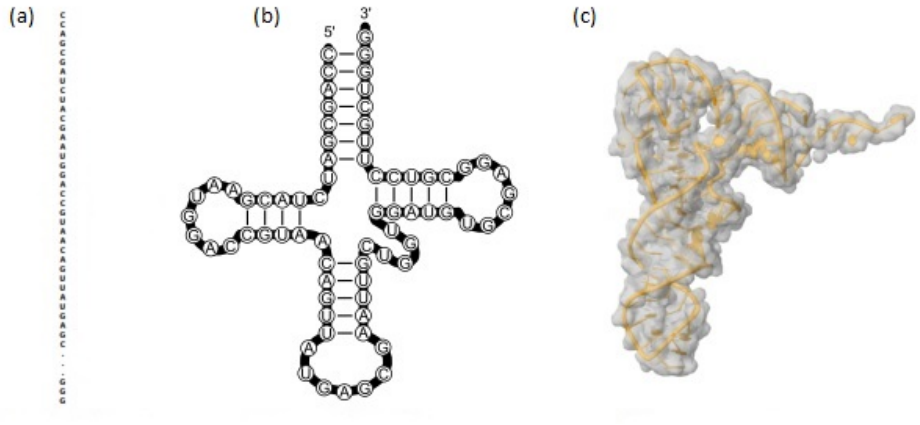


Figura 2.11: Representação das estruturas de ncRNAs (adaptado de [59], respectivamente, (a) estrututra primária; (b) estrutura secundária e (c) estrutura terciária.

Capítulo 3

Sistemas Multiagentes e o ncRNA-Agents

Este capítulo contém os conceitos básicos de agentes (Seção 3.1) e sistemas multiagentes (Seção 3.2). Na Seção 3.3, serão descritas as ferramentas usadas para a construção do ncRNA-Agents. Em seguida, na Seção 3.4 será descrito em detalhes o ncRNA-Agents, foco deste projeto.

3.1 Agentes

Ainda não existe uma definição aceita universalmente sobre o que são agentes, pelo contrário, o debate ainda é muito intenso. Para estabelecer algum tipo de ordem adotaremos uma definição simples de agente inteligente: Agente inteligente é uma entidade (computacional ou não) autônoma capaz de perceber o ambiente ao qual está inserido e agir sobre o mesmo, a fim de satisfazer determinados objetivos [67]. A percepção é feita por meio de sensores e as ações são realizadas por meio de atuadores, como mostra a Figura 3.1.

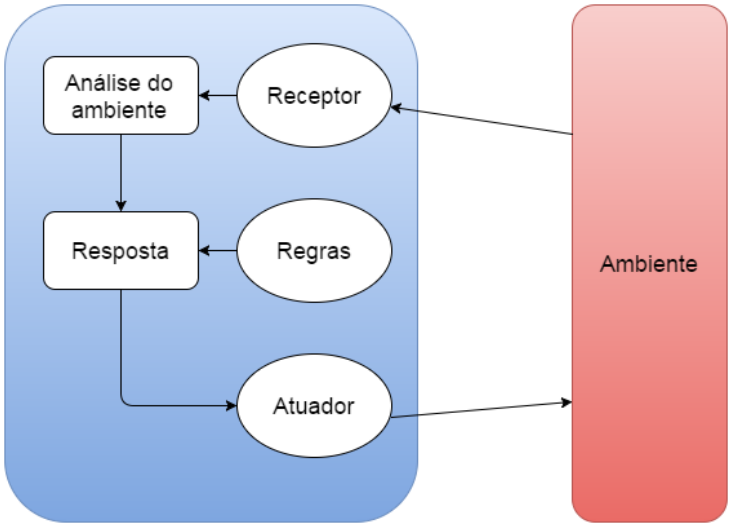


Figura 3.1: Representação de um agente inteligente (adaptado de [67]).

Um agente computacional é capaz de executar operações requisitadas por um usuário ou outro programa, com certo grau de independência ou autonomia e, ao fazê-las, emprega algum conhecimento dos objetivos ou desejos do usuário. As principais caracteristicas de um agente inteligente, de acordo com [67], são:

- Autonomia: os agentes inteligentes são capazes de trabalhar de maneira independente no ambiente em que estão inseridos, aprender por experiência e alterar seu comportamento;
- Reatividade: os agentes inteligentes são capazes de perceber seu ambiente, e responder em tempo hábil às mudanças que ocorrem nele, a fim de satisfazer os objetivos de projeto;
- Proatividade: os agentes inteligentes podem mostrar um comportamento com foco em propósitos, reconhecendo oportunidades e tomando inciativa, a fim de satisfazer seus objetivos;
- Sociabilidade: os agentes são capazes de interagir com outros agentes e ambientes a fim de satisfazer seus objetivos, através de protocolos de comunicação.

O ambiente em que se localiza pode exigir mais ou menos racionalidade do agente. Os tipos básicos de agentes que incorporam os princípios de sistemas baseados em agentes inteligentes são [47]:

- Reativo simples: é o agente mais simples pois ele escolhe uma ação pré-determinada para atuar no ambiente em que está inserido, de acordo com suas percepções;
- Reativo baseado em modelo: é capaz de lidar com ambientes parcialmente observáveis. Possui um estado interno baseado no histórico das percepções de maneira a conseguir refletir os aspectos não observados pelo estado atual, sua percepção vigente é combinada com um estado interno salvo anteriormente para gerar a descrição atual do ambiente;
- Baseado em objetivo: a partir de um estado interno, possui um conjunto de objetivos alcançáveis. As ações são tomadas baseadas nos objetivos e levando em consideração o ambiente em que o agente se encontra;
- Baseado em utilidade: procura atingir um grau de satisfação com o mundo em que se encontra, isto é, o agente procura a "felicidade" de acordo com uma função de utilidade, que irá atribuir um determinado grau de satisfação ("feliz" ou "infeliz") ao agente no momento;
- Baseado em aprendizado: são mantidas as percepções passadas do ambiente e as que poderão ser utilizadas. Possui um *feedback* crítico que indica como o agente esta atuando no ambiente e determina suas modificações para atuar no futuro.

3.2 Sistemas Multiagentes

Um Sistema Multiagente (SMA) inclui diversos agentes que interagem ou trabalham em conjunto, podendo compreender agentes de tipo homogêneo ou heterogêneo. Cada agente opera assincronamente com respeito aos outros agentes [67].

Para que um agente possa operar como parte do sistema, faz-se necessária a existência de uma infraestrutura que permita a comunicação e interação entre os componentes do SMA, podendo ser feita de forma direta (comunicação explícita) ou de modo indireto (emissão de sinais através do ambiente) [3].

Pode-se definir um sistema como sendo multiagente quando ele possui determinadas características: um ambiente, um conjunto de agentes, um conjunto de objetos, as interações entre os próprios agentes e um conjunto de operações que podem ser realizadas [3].

Assim, dado um SMA, denomina-se agente a cada uma das suas entidades ativas no sistema. O conjunto formado pelos agentes é chamado de sociedade. O termo ambiente representa as entidades passivas do sistema. Um agente recebe informações e raciocina sobre o ambiente, sobre outros agentes e decide quais ações deve realizar e quais objetivos deve seguir.

A Figura 3.2 mostra que adotar uma estratégia orientada a agentes viabiliza a decomposição do problema em componentes múltiplos e autônomos, os quais podem interagir de forma flexível para alcançar seus objetivos, conforme interação entre organizações de agentes com diferentes esferas de inuência no ambiente.

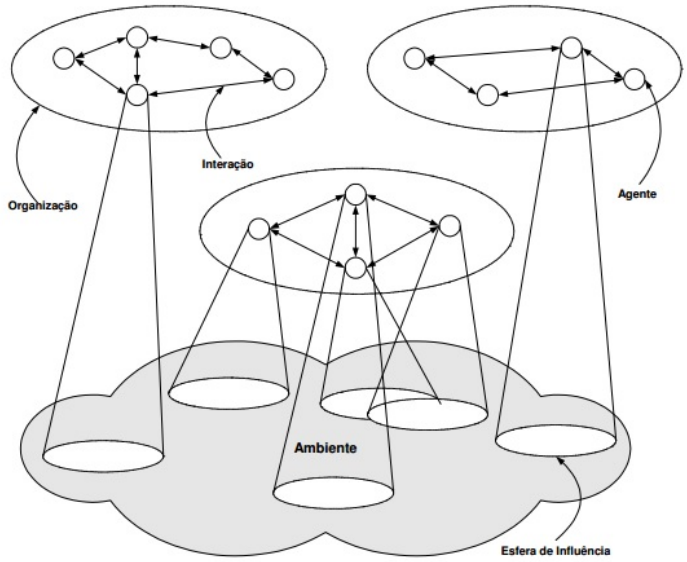


Figura 3.2: Representação de um sistema multiagente (adaptado de [67]).

Os SMAs costumam ser divididos em duas classes: a primeira é denominada SMA Reativo, uma classe que trabalha com o desenvolvimento de sistemas que utilizam um grande número de agentes simples, os quais buscam a resolução de um determinado problema; já a segunda abordagem, denominada SMA Cognitivo, trabalha com poucos agentes que buscam resolver tarefas mais complexas que os reativos. No entanto, podem existir sistemas híbridos, onde coexistem os dois tipos de agentes.

Sistema Multiagente Reativo

A idéia principal no SMA reativo é que um comportamento global inteligente possa ser alcançado a partir do comportamento individual dos agentes. Esse sistema é baseado em modelo de organização biológica ou etológica (comportamental) tais como: formigas,

cupins, abelhas, entre outros. O Sistema Multiagente Reativo funciona por meio da ação-reação.

Para o melhor entendimento vamos ao exemplo clássico de uma colônia de formigas: uma formiga isoladamente possui poucas capacidades, parece um ser não muito inteligente, agora já o comportamento de uma colônia de formigas, como um todo, é bem estruturado e fez com que as formigas sobrevivessem por milhões de anos.

O SMA reativo concebe o problema como sendo um conjunto de agentes interagindo/comunicando entre si, onde cada um destes possui seus objetivos individuais.

As principais características de um SMA reativo são:

- Conhecimento do ambiente não representado de forma explícita: o agente tem seu conhecimento implícito em regras de comportamento e sua manifestação se expressa através do seu comportamento em interação com o ambiente e/ou outros agentes;
- Estado do ambiente não representado internamente: o comportamento de cada agente tem como base sua percepção dos estímulos recebidos do ambiente, mas o agente não tem representação interna explícita do estado do ambiente;
- Registro/memória das ações inexistentes: não mantém um histórico das ações, assim, o resultado de uma determinada ação passada não irá influenciar diretamente na decisão de uma ação que possa ocorrer no futuro;
- Organização etológica: é similar à observada por animais que vivem em grandes comunidades, como formigas e cupins;
- Grande quantidade de membros: em geral, possuem um grande número de agentes, com populações que podem alcançar milhares de membros.

Sistema Multiagente Cognitivo

Os agentes cognitivos podem interagir com os demais membros da sua comunidade através de linguagens e protocolos de comunicação, utilizando-se de estratégias sofisticadas de negociação. O SMA cognitivo é baseado em modelos de organização social de sociedades humanas, como: grupos, hierarquias, mercados.

Esse tipo de agente possui uma representação concreta do ambiente e dos membros que estão na comunidade e podem raciocinar sobre as ações que foram tomadas no passado e assim planejar as ações a serem tomadas no futuro.

As principais características associadas ao SMA cognitivos são [3]:

- Estado do ambiente e de outros agentes representados de forma **explícita** e agentes que podem **interagir** entre si, em sociedade;
- Possui memória: A memória, permite **planejar** suas ações futuras, devido a sua capacidade de **lembrar** de ações passadas;
- Comunicação Direta: utiliza a percepção para examinar o ambiente, e o mecanismo de comunicação que permite a troca de mensagens entre os agentes. A comunicação entre os agentes pode ser feita de modo direto, com o envio e o recebimento de mensagens;

- Agentes cognitivos podem **raciocinar** e decidir em conjunto sobre quais ações devem executar, quais planos seguir e quais objetivos devem alcançar;
- Baseiam-se em modelos sociológicos, como organizações humanas;
- Poucos Agentes: Usualmente um SMA cognitivo contém algumas dezenas de agentes.

A Tabela 3.1 mostra uma comparação entre SMAs reativos e cognitivos.

Tabela 3.1: Comparação entre os tipos de SMAs.

Reativo	**Cognitivo**
representação implícita	representação explícita
comunicação indireta	comunicação direta
controle não deliberativo	controle deliberativo
organização etológica	organização social
muitos agentes	poucos agentes

3.2.1 Caracterização do Ambiente

Em Weyns e Holvoet [66], a definição de ambiente é dada como uma abstração de primeira classe que fornece as condições para os agentes existirem. Além disso o ambiente controla o acesso entre os agentes e o acesso aos recursos. Os receptores do agentes captam as informações fornecidas pelo ambiente. São classificações dos ambientes [47] (classificados quanto a suas propriedades):

- Acessível ou inacessível: o ambiente acessível é aquele no qual o agente pode obter informações completas, precisas e atualizadas sobre o seu estado. Quanto mais acessível um ambiente for, mais simples será o projeto e a construção do agente. Mas a maioria dos ambientes de mundo real não é totalmente acessível. Caso um ambiente não seja acessível, é considerado inacessível;
- Determinístico ou não-determinístico: o ambiente determinístico é aquele no qual uma ação possui um único efeito possível. Quando não é possível prever o estado que o ambiente assumirá após a execução de uma ação, dizemos que o ambiente é não-determinístico. O não-determinístico pode incluir incertezas sobre os estados resultantes da execução de ações;
- Estático ou dinâmico: o ambiente é estático para um agente quando permanece inalterado até o momento em que ele executa alguma ação. Em um ambiente dinâmico, além das ações desempenhadas pelos agentes, existem processos que operam sobre o ambiente, alterando o estado do mesmo de forma dinâmica;
- Discreto ou contínuo: o ambiente discreto é aquele que possui um número fixo ou finito de estados a serem percebidos e correspondentes ações. Um ambiente contínuo é aquele no qual é possível assumir um número infinito de estados.

3.2.2 Protocolo de Comunicação

Um agente inteligente deve possuir a habilidade para interagir e comunicar com outros agentes, que estão no ambiente em que se insere. Sendo assim, uma característica fundamental do SMA é a comunicação. Conforme Russell e Norvig [47], **comunicação** é a troca de informação feita pelos agentes de forma intencional, com percepção de sinais extraídos de um ambiente compartilhado.A comunicação tem duas finalidades principais:

1. Compartilhamento do conhecimento, informações, crenças com os outros agentes;
2. Coordenação das atividades entre os agentes [3].

Então, para que haja comunicação que permita atinja essas duas metas, como mostrado na Figura 3.3, faz-se necessário a definição de algum tipo de linguagem comum ou compartilhada com os agentes que estão no ambiente. Para tal, uma linguagem de comunicação deve possuir as seguintes características:

- Sintaxe: é a relação existente entre as palavras dentro de uma unidade, usando a gramática que contém as regras que são relativas à um conjunto/combinação de palavras em unidades maiores;
- Semântica: é a combinação feita com símbolos e seus respectivos significados, busca-se fazer um estudo sobre o significado para as palavras e dos enunciados;
- Vocabulário: explicação sucinta com definições de uma lista de vocábulos de linguagem, que em geral estão desacompanhadas de sua respectiva definição;
- Pragmática: é o conjunto de regras de ações que define para que a interpretação dos símbolos através da comunicação é feita;
- Modelo de domínio de discurso: é o significado que um conjunto de símbolos que pode assumir depois da interpretação feita dentro de um contexto específico [3].

3.2.3 Protocolo de Interação

Protocolos de interação são usados para especificar o comportamento entre os agentes durante a interação. Com o intuito de garantir uma forma efetiva de operação, faz-se necessário a definição de um protocolo de comunicação e interação.

Um protocolo de interação pode especificar que, quando um agente receber uma mensagem *Request* com um dado pedido, só poderá responder com a mensagem *Not-Understood* (se não percebe algum aspecto da mensagem recebida), ou com a mensagem *Refuse* (se não aceita o pedido que lhe é feito), ou com a mensagem *Agree* (se aceita o pedido que lhe é feito) [3].

A utilização de protocolos de interação auxiliam no projeto de aplicações baseadas em agentes pois permite reduzir o número de alternativas que um agente deve considerar em cada passo da sua interação com outros.

Um importante protocolo usado no ambiente de negociação é o protocolo *Contract net* [52]. O *Contract net* usa uma estrutura descentralizada de negociação, onde seus agentes podem demandar serviços, recursos ou informações para outros agentes.

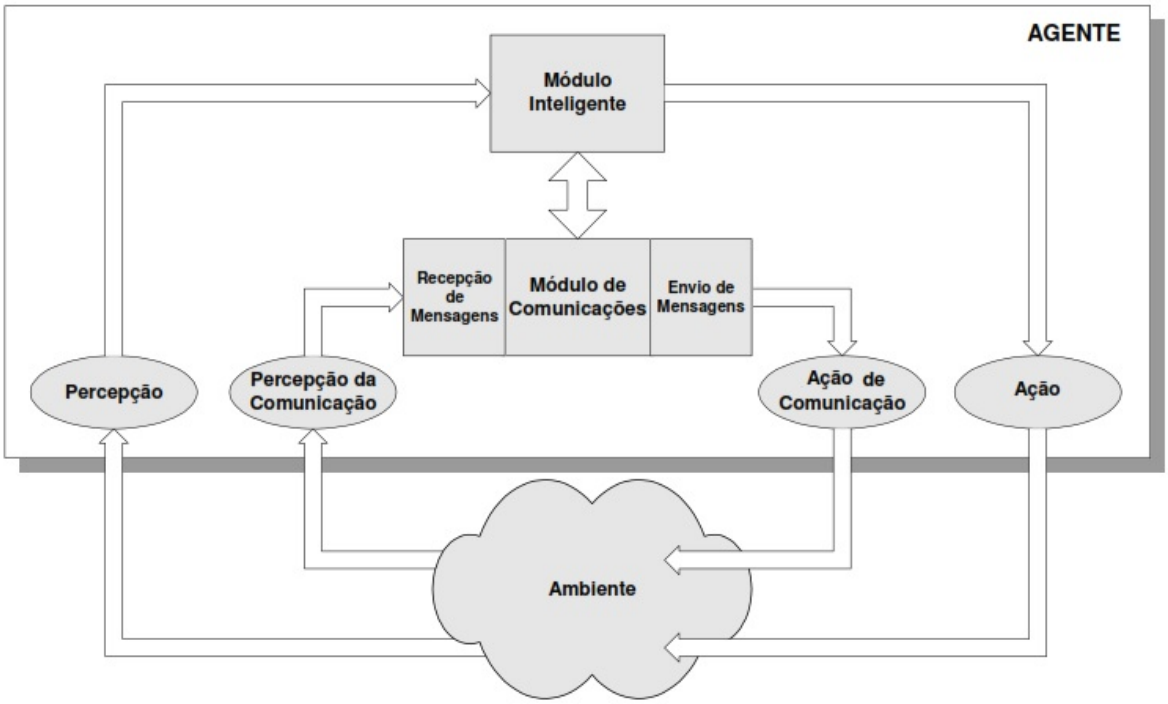


Figura 3.3: Agente com Capacidade de Comunicação (adaptado de [67]).

A principal ideia então é a de que, quando um agente não consegue resolver um problema sozinho, ele irá decompor seus problemas em subproblemas e encontrará outros agentes para auxiliá-lo na resolução de cada subproblema. Tal abordagem é conhecida como abordagem de dividir para conquistar.

A definição do protocolo de interação e do protocolo de comunicação auxiliam no processo de interação dos agentes viabilizando diálogos para o alcance de objetivos.

3.3 Ferramentas

Nesta seção serão descritas as ferramentas e bancos de dados utilizadas para a construção do ncRNA-Agents que será detalhado mais a frente.

3.3.1 Ferramentas para construir SMAs

Aqui se encontra uma descrição do JADE [22], um *framework* de desenvolvimento de Sistemas Multiagentes, e do Drools [18], que permite inferências em bases de conhecimento.

JADE

JADE *(Java Agent Development Framework)* [22] é um *middleware* para o desenvolvimento em tempo de execução de aplicações *peer-to-peer* que se baseiam no paradigma dos agentes e que pode interoperar, tanto em ambiente com fio quanto em ambientes e sem fio. O JADE facilita o desenvolvimento de sistemas multiagentes.

A plataforma JADE foi inicialmente desenvolvida pelo grupo de pesquisa e desenvolvimento da Telecom Itália em parceria com a Universidade de Parma. Entretanto, há alguns anos passou a ser um projeto da comunidade e tornou-se uma tecnologia *open source* (sob as normas da licença *Lesser General Public License* (LPGL)), dentre as mais comuns em utilização hoje. Possui suporte para o desenvolvimento de aplicações, que possui o agente de software como abstração.

Como uma linguagem de programação, seus principais concorrentes são Java e C #, enquanto como um banco de dados compete com outros bancos de dados orientados a objetos e bancos de dados pós-relacionais, como Versant, Caché e Matisse, bem como pacotes de software de banco de dados relacionais tradicionais, tais como Oracle e Microsoft SQL Server.

Drools

O Drools [18] é uma ferramenta para construção de bases de conhecimento e de inferência dirigida por padrões. Foi construído para interagir com Java e o conhecimento é obtido de regras declarativas. Uma regra Drools tem uma ou mais condições (ou fatos) que levam a uma ou mais ações (ou consequências).

Basicamente, o motor de inferência do Drools oferece a possibilidade de utilização do método de encadeamento progressivo e regressivo. O algoritmo de inferência presente no Drools é o RETE [23], sendo adaptado para sistemas orientados a objetos.

Basicamente o Drools permite elaborar regras de negócio declarativas, separar e centralizar as regras de negócio de uma aplicação, e fazer o gerenciamento das regras alterando-as e mudando suas versões dinamicamente [3]. Ele é composto por [18]:

1. Uma máquina de inferência, que é responsável pela execução das regras;
2. Uma memória de trabalho, utilizada para armazenar os fatos gerados pela execução das regras;
3. Uma base de conhecimento, que é o local onde estão contidas as regras que o mecanismo de inferência vai utilizar.

A Figura 3.4, mostra o diagrama com os elementos que compõem o Drools.

3.3.2 Ferramentas para anotar ncRNAs

Aqui serão descritos os programas, bancos de dados e pacotes que serão utilizados no sistema de anotação de ncRNAs.

BLAST

O BLAST (*Basic Local Alignment Search Tool*) [48] é utilizado para se realizar a comparação aproximada entre duas sequências. É um método de alinhamento local, que compara sequências com funções já definidas e armazenadas em um banco de dados. O BLAST pode ser utilizado para identificar relações evolucionárias, inferir funções ou identificar uma família de genes entre sequências [48].

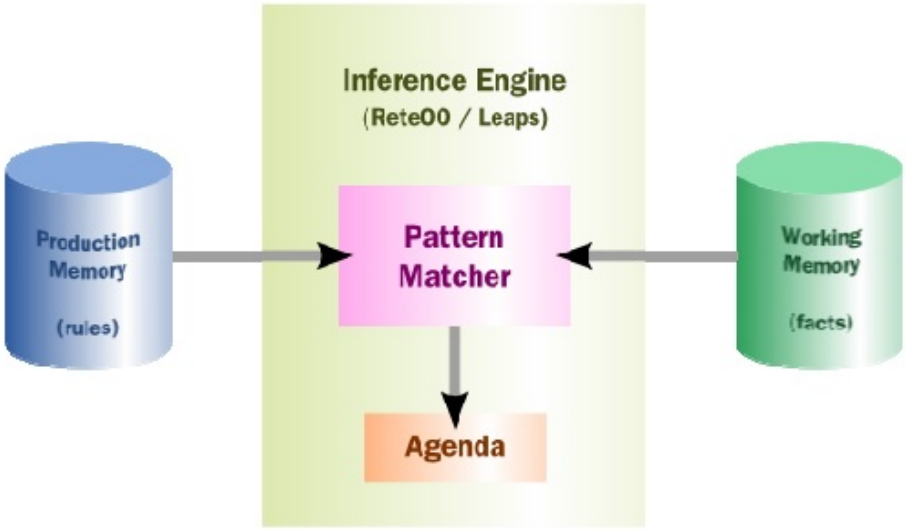


Figura 3.4: Diagrama com elementos que compõem o Drools [18].

O BLAST é constituído por vários programas a depender do tipo da sequência a ser comparada e das sequências armazenadas no banco de dados, podem ser bases (DNA) ou aminoácidos (proteínas):

1. BLASTp: comparação de sequências de aminoácidos com um banco de dados de aminoácidos;
2. BLASTn: comparação de sequências de nucleotídeos com um banco de dados de nucleotídeos;
3. BLASTx: comparação de sequências de nucleotídeos traduzidos em ORFs (*open reading frame*), com um banco de dados de aminoácidos;
4. tBLASTn: comparação de sequências de proteínas com um banco de dados de sequências de nucleotídeos traduzidos em ORFs;
5. tBLASTx: comparação de ORFs de sequências de nucleotídeos com todas as ORFs de um banco de dados de nucleotídeos.

Infernal

O Infernal (*INFErence of RNA Alignment*) [3] utiliza uma abordagem baseada em Gramática Estocástica Livre de Contextos (SCFG, *Stochastic Context-Free Grammars*). Essa ferramenta é capaz de construir perfis de **RNA consenso** com base em Modelos de Covariância (*Covariance Models*-CM), que são casos especiais de SCFG, projetada para modelar sequências e estruturas de RNA. Utilizando-se do banco de dados Rfam [9] (descrito mais adiante), a ferramenta procura semelhança entre estruturas secundárias das famílias de RNAs e a sequência investigada.

SVM- Portrait

Máquinas de suporte vetorial (SVM) [30] são um grupo de métodos de aprendizagem supervisionados que podem ser aplicados à classificação ou regressão. As máquinas de vetores de suporte representam uma extensão de modelos não-lineares do algoritmo de retrato generalizado. São formados grupos que são classificados com base na criação de margens e na separação de tais margens. Eles então são delineadas por uma fração dos dados de treinamento, os vetores de suporte.

O Portrait [44] por sua vez, identifica ncRNAs de transcritomas não completos de espécies que ainda não estão totalmente caracterizadas, com base nas máquinas de suporte vetorial. Seu resultado é uma probabilidade indicando a quais as chances de uma transcrição ser não codificadora de proteína.

tRNAscan-SE

O tRNAscan-SE [3] combina três programas: os dois primeiros preditores de tRNAs e o terceiro, um CM treinado com sequências de tRNAs. Os dois primeiros programas são rápidos, mas acarretam em uma grande taxa de falsos positivos, enquanto o CM é lento, mas muito sensível e específico. Assim, os dois primeiros programas são utilizados como um filtro para que só os candidatos filtrados passem a ser analisados pelo Modelo de Covariância.

Vienna

O pacote Vienna [35] consiste de uma biblioteca de código C e vários programas autônomos para a previsão e comparação de estruturas secundárias de RNA. Suas ferramentas são capazes de realizar os dobramentos dos RNAs utilizando um algoritmo de predição baseado na energia livre do RNA, e nas probabilidades de pareamentos de bases.

A predição da estrutura secundária do RNA através da minimização da energia é a função mais utilizada no pacote. São três tipos de algoritmos de programação dinâmica para a previsão da estrutura: o algoritmo de energia livre mínimo de Zuker e Stiegler 1981, que produz uma única estrutura ótima, o algoritmo de função de partição de McCaskill, 1990, que calcula probabilidades de pares de bases no conjunto termodinâmico, e o algoritmo de dobramento sub-ótimo de Wuchty, 1999, que gera todas as estruturas sub-ótimas dentro de uma dada faixa de energia da energia ótima. Para a comparação da estrutura secundária, o pacote contém várias medidas de distância (dissimilaridades) usando alinhamento de cordas. Existe também um algoritmo para projetar sequências com uma estrutura predefinida (dobra inversa).

Em particular, temos os pacotes RNAfold [35] e RNAz [35] que são de grande importância para este trabalho. O RNAfold é baseado na hipótese de que uma molécula de RNA é dobrada em uma estrutura termodinâmica estável com mínima energia livre.

Já o RNAz é um pacote de software amplamente utilizado para a detecção *de novo* ncRNAs em dados de comparação genômica. A ferramenta detecta ncRNAs por meio de uma abordagem comparativa. Além de medir a conservação evolutiva, também avalia explicitamente a estabilidade termodinâmica da estrutura secundária. Uma máquina de vetor de suporte (SVM) é então usada para avaliar ambos os critérios.

O RNAz foi utilizado com sucesso para mapear ncRNAs estruturais em uma grande variedade de genomas. Um grande número dessas previsões também foram verificados experimentalmente. A grande utilização do RNAz também abriu portas para a identificação de falhas e melhorias que são necessárias.

SnoReport

O snoReport [2] tem a proposta de identificar duas classes principais de *small nucleolar* RNAs (snoRNAs), que são o H/ACA box e o C/D box. Combina a aprendizagem de máquina com a predição da estrutura secundária, utilizando uma máquina de vetores de suporte (SVM) [30] e utiliza emprego do método máquina de vetor de suporte (*Support Vector Machine - SVMS*) como mecanismo de aprendizagem de máquina, combinado com a ferramenta RNAfold, do pacote Vienna, para predição de estrutura secundária.

O conjunto de treinamento do snoReport, é composto de amostras positivas (sequências das principais classes de snoRNAs, retiradas do banco de dados snoRNABase), e amostras negativas (conjunto de sequências que não são snoRNAs, retirados do miRBase, Rfam e de um conjunto aleatório de sequências). O conjunto de amostras positivas é frequentemente pequeno [25].

Clustal Ômega

O Clustal Ômega [11] pode alinhar praticamente qualquer número de sequências de proteínas rapidamente e fornece alinhamentos precisos. A precisão do pacote em casos de teste menores é semelhante à dos alinhadores de alta qualidade. Em conjuntos de dados maiores, o Clustal Ômega supera outros pacotes em termos de tempo de execução e qualidade. Além disso, a ferramenta também tem recursos poderosos para adicionar sequências e explorar informações em alinhamentos existentes, fazendo uso da grande quantidade de informações pré-computadas em bancos de dados públicos.

Segemehl

Segemehl é um software para mapear sequências curtas a um genoma de referência. Diferentemente de outros métodos, o Segemehl é capaz de detectar não apenas incompatibilidades, mas também inserções e deleções. A ferramenta implementa uma estratégia de correspondência baseada em vetores de sufixos. O Segemehl agora suporta o formato SAM, lê consultas *gziped* para economizar espaço em disco e memória.

3.3.3 Bancos de Dados

Abaixo encontra-se uma breve descrição dos bancos de dados utilizados no ncRNA-Agents:

- **NONCODE** [42]: Todos os ncRNAs utilizados pelo NONCODE foram filtrados de maneira automática do GenBank e da literatura e são tratados manualmente;
- **RNAdb** [45]: Contém sequências e anotações de ncRNAs de mamíferos, mas em grande parte com ncRNAs com funções ainda desconhecidas;
- **miRbase** [41]: Banco de dados contendo miRNAs;

- **snoRNA Database** [54]: Contém snoRNAs humanos do tipo H/ACA *box* e C/D *box*;
- **Plant snoRNAs Database** [53]: Contém snoRNAs de plantas;
- **Rfam** [9]: É uma base de dados curada (revisada e supervisionada) que contém informações de milhares de famílias de ncRNAs.

3.4 NcRNA-Agents

Anotar sequências significa descobrir suas funções biológicas, essa tarefa pode ser executada por algoritmos de comparação de sequências (por homologia) ou por extração de características (como tamanho da sequência ou *open reading frame* (ORF)).

O ncRNA-Agents utiliza uma classificação dos métodos computacionais para anotação em três paradigmas:

Homologia

Visa a predição de ncRNAs por meio de comparação de sequências de duas ou mais espécies. As predições dependem de banco de dados curados, isso implica que, quanto melhores as anotações, melhores serão as predições.

Genes são homólogos quando possuem um ancestral em comum,o que permite que esses genes mantenham a mesma funcionalidade herdada. Sequências homólogas dividem-se em duas classes: ortólogas e parálogas. As ortólogas são sequências relacionadas por especiação, possuindo uma descendência vertical, já as parálogas são sequências relacionadas por duplicação, dentro da mesma espécie ou nos ancestrais [3].

Exemplos de ferramentas utilizadas para a predição de homologia são o BLAST [48], o tRNAscan-SE [3] e o Infernal [3] (serão descritos mais a frente). A métrica a similaridade de sequências (quanto mais parecidas as duas sequências, maior a chance delas terem herdado a mesma função). O BLAST não possui um bom desempenho em descobrir ncRNAs, enquanto o Infernal é mais sensível e específico para anotar ncRNAs.

Predição de Classe

Inclui ferramentas baseadas nos métodos de aprendizagem de máquina. No aprendizado supervisionado, pode-se tomar um conjunto conhecido de ncRNAs e um conjunto conhecido de proteínas, calculando características *ab initio* [3] dessas sequências, visando criar um modelo de predição de ncRNAs. Confere ao modelo uma maior confiabilidade. A ferramenta uitlizada para a predição de classe é o SVM-Portrait [30].

Modelos *De Novo*

Classe que inclui ferramentas para anotação de ncRNAs criadas a partir de outros modelos, diferentes de homologia e predição de classe.

Um exemplo que pode ser citado é o modelo termodinâmico, no qual ordem dos nucleotídeos na sequência primária e as possibilidades de pareamento em uma molécula de RNA de maneira a diminuir a energia livre da estrutura são usadas para predizer forma espacial. Uma investigação dessa forma resulta em um conhecimento similar sobre suas

propriedades fisiológicas. A ferramenta baseada em modelo termodinâmico utilizada pelo ncRNA-Agnets é o Vienna [35], que será descrito a seguir.

Com base nesses paradigmas, foi especificado o projeto multiagente correspondente, por meio das ferramentas descritas na Seção 3.3.

3.4.1 Arquitetura

Arruda *et. al.* [3] propuseram a arquitetura conforme apresentado na Figura 3.5 para o ncRNA-Agents. As camadas são descritas a seguir.

Descrição das Camadas

- Camada de Interface recebe uma requisição do usuário, composta por uma ou mais sequências, em formato *FASTA*, e pela seleção de um conjunto de ferramentas de anotação. Após o processamento, a camada retorna, para cada sequência, a anotação sugerida, juntamente com os resultados e cálculos feitos pelas ferramentas que foram utilizadas no processo de raciocínio;
- Camada de Resolução de conflitos decide qual é a melhor recomendação para a anotação de cada sequência, a partir das diversas sugestões recebidas da camada colaborativa. É onde é simulado o raciocínio biológico;
- Camada Colaborativa é responsável pela execução das diversas ferramentas (escolhidas pelo usuário) para anotar ncRNAs, e pela análise e inferência dos seus resultados. Em seguida envia os resultados filtrados para a camada de resolução de conflitos;
- Camada Física inclui os bancos de dados.

Foram utilizadas algumas regras na Camada de Resolução de Conflitos (Figura 3.6), que simulam o raciocínio dos biólogos para analisar os resultados obtidos de ferramentas das três classes propostas e na investigação entre organismos relacionados.

Descrição dos Agentes

A camada colaborativa possui dois tipos de agentes: Agentes Gerentes e Agentes Analistas.

Agentes Gerentes realizam análises e inferências nas sugestões enviadas pelos Agentes Analistas, e também simulam o raciocínio dos biólogos [3]. Existem cinco tipos de Agentes Gerentes, três baseados nas classes Homologia, Modelos de Novo e Predição de Classe, e três outros gerentes criados para avaliar a anotação recomendada. Esses dois últimos tem a função de remover falsos positivos, ou procurar novos ncRNAs, encontrados a partir da conservação da sequência em organismos relacionados. Abaixo fazemos uma breve descrição dos cinco tipos de Agentes Gerentes:

- Agente Gerente de Homologia comanda agentes que trabalham com ferramentas baseadas em homologia;
- Agente Gerente de Predição de Classe comanda os agentes que trabalham com ferramentas baseadas em Aprendizagem de Máquina;

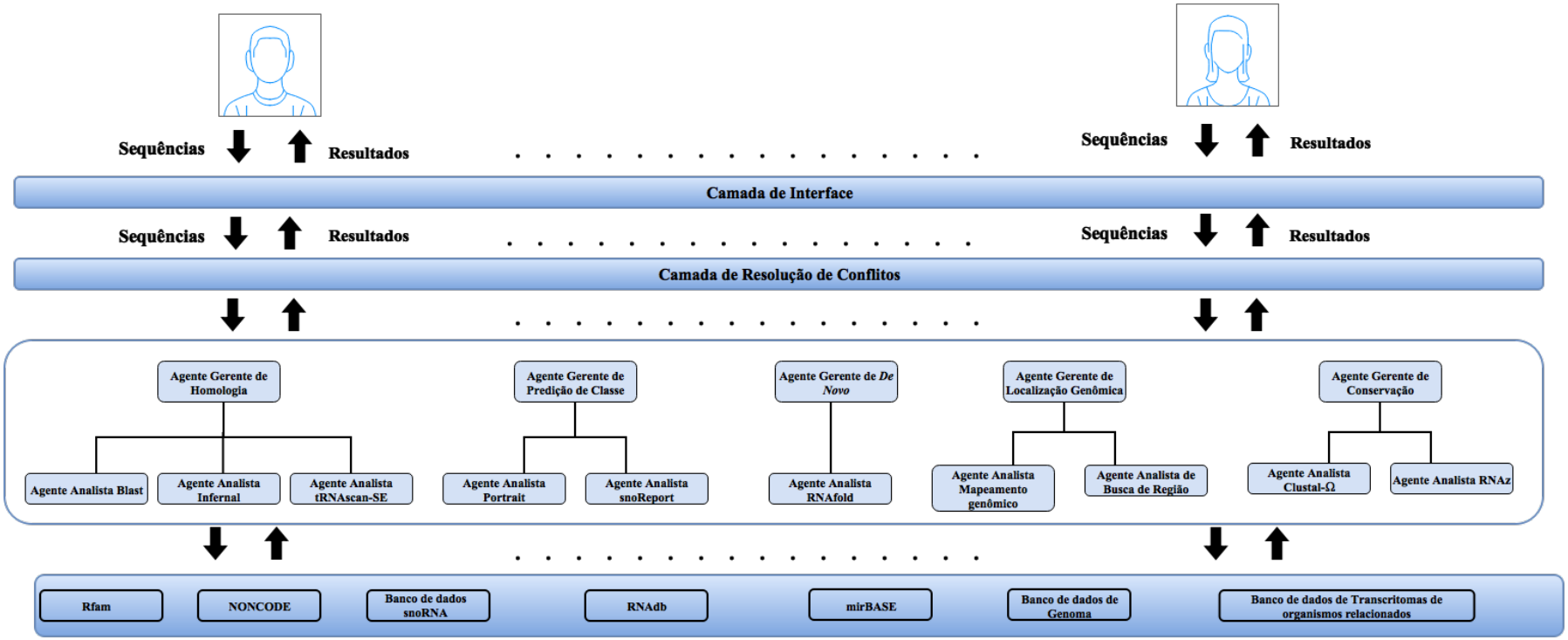


Figura 3.5: Arquitetura do ncRNA-Agents [3].

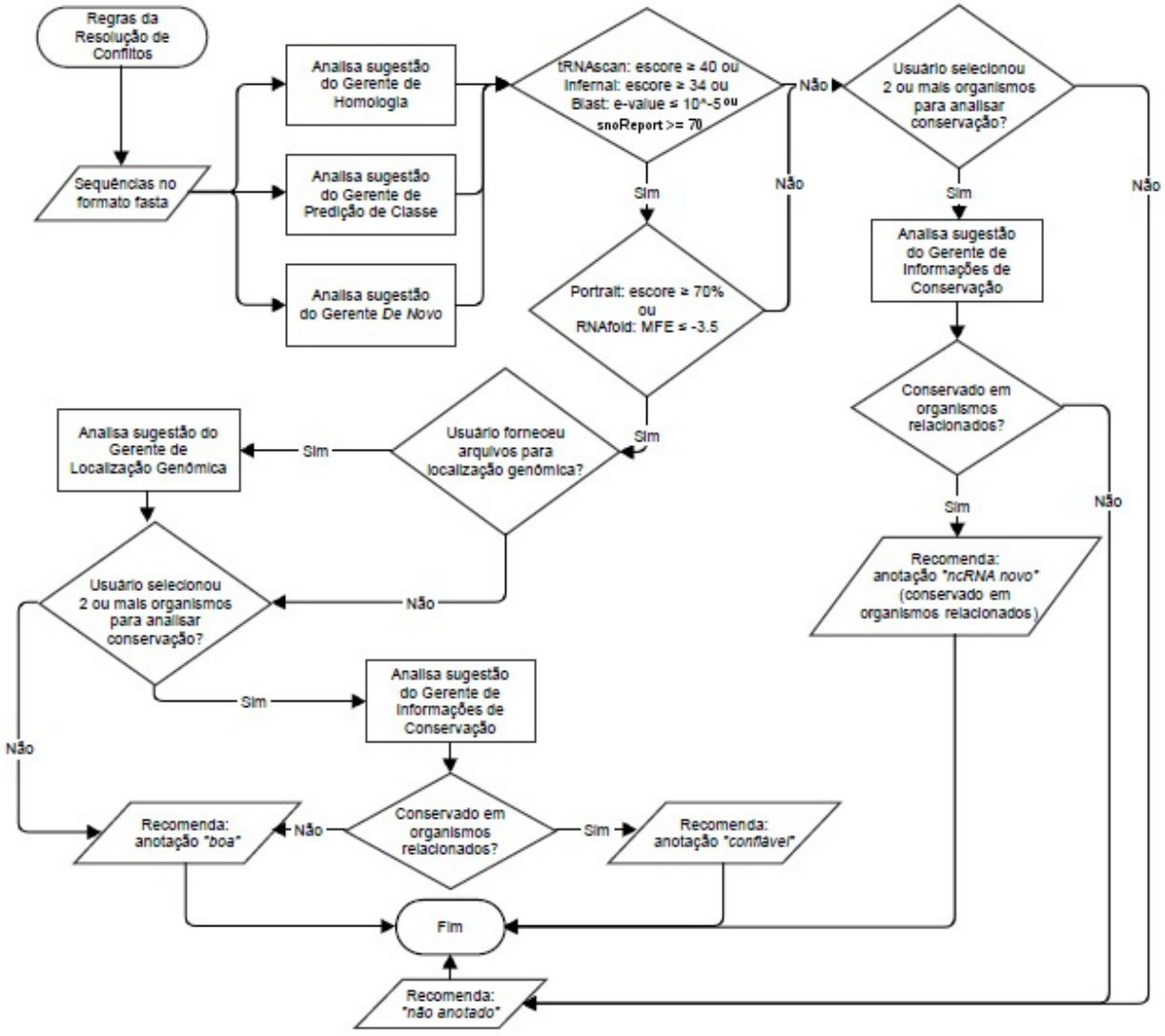


Figura 3.6: Regras utilizadas na Camada de Resolução de Conflitos, que simulam o raciocínio dos biólogos para analisar os resultados obtidos de ferramentas das três classes propostas e na investigação entre organismos relacionados [3].

- Gerente De Novo coordena agentes que trabalham com ferramentas que realizam e buscam informações a partir da própria sequência;
- Agente Gerente de Localização Genômica comanda agentes que trabalham com ferramentas de alinhamento, resultantes de métodos de comparação de sequências, com o intuito de descobrir se uma sequências pode ser mapeada em um genoma de referência, o que permite verificar se a sequência é de fato um ncRNA;
- Agente Gerente de Conservação gerencia agentes que trabalham com ferramentas que investigam conservação entre os organismos relacionados.

Os Agentes Analistas são responsáveis por executar ferramentas especifícas para análise de ncRNAs. Cada Agente Analista, criado por solicitação de um agente gerente, executa uma análise *(parse)* para extrair informações do arquivo de saída criado pela ferramenta específica controlada por ele.

3.4.2 Detalhes de Implementação

O ncRNA-Agents foi implementado utilizando o Drools 6.1.0 e o *Framework* JADE versão 4.3.33 para simular o raciocínio dos agentes. Foram utilizadas regras declarativas para a formalização do conhecimento biológico.

Para o Gerente de Homologia, as regras foram criadas para analisar os resultados da suas ferramentas, BLAST, Infernal e tRNAscan. O melhor resultado é enviado para a Camada de Resolução de Conflitos. O tRNAscan verifica se existem bons alinhamentos, escolhendo o melhor escore entre eles. O BLAST procura por um alinhamento com *e-value* $\leq 10^{-5}$. Já o Infernal analisa o alinhamento com escore ≥ 34. A recomendação de anotação segue a ordem de resultados dados por Infernal, tRNAscan e BLAST, caso algum resultado seja encontrado de acordo com os valores pré-definidos.

Interface

A interface com o usuário [3], criada através de um projeto Web, pode ser vista na Figura 3.7. A interface permite ao usuário informar as sequências no formato *FASTA* e definir os parâmetros de execução, tais como ferramentas e banco de dados de ncRNAs. Após a configuração feita pelo usuário, a validação dos parâmetros selecionados é realizada e, em caso de sucesso, uma requisição será criada e submetida para o sistema.

Caso a requisição tenha parâmetros inválidos, uma mensagem de erro será exibida para o usuário, destacando os campos onde foram encontrado erros. A interface também permite que o usuário acesse uma requisição já existente, na aba *Fetch request*, que tenha o status de concluída ou em andamento. A aba *Case Study* fornece a opção de consultar estudos de caso já realizados.

Com a requisição submetida, a interface espera pela primeira resposta do ncRNA-Agents que, quando recebida, redireciona o usuário para a página com os resultados. Na página de resultados, uma mensagem informativa é apresentada ao usuário para lembrá-lo de guardar o identificador da requisição para posteriores consultas. Caso um arquivo possua múltiplas sequências, será apresentados ao usuário os resultados obtidos para todas as sequências. Caso necessário, os resultados podem ser mostrando em várias abas, conforme a quantidade de sequências submetidas.

Figura 3.7: Interface do ncRNA-Agents.

3.4.3 Resultados gerados pelo ncRNA-Agents

O ncRNA-Agents exibe os resultados produzidos em uma página web, conforme mostrado na Figura 3.8. Se o usuário precisar de mais detalhes, pode solicitar informações mais detalhadas clicando no *link* de interesse, e obtendo mais resultados como mostrados nas Figuras 3.9 a 3.13.

Qualidade de Anotação

Os resultados encontrados no ncRNA-Agents recebem uma chamada qualidade de anotação conforme consta na Figura 3.6. A Tabela 3.2 mostra as indicações de qualidade de anotação, criadas a partir das classes de ferramentas propostas no ncRNA-Agents.

Tabela 3.2: Avaliação da qualidade das anotações (adaptado de [3]).

Qualidade da Anotação	**Razão**
boa	Anotação por homologia, confirmada por predição de classe e/ou *de Novo*
confiável	Anotação por homologia,confirmada por predição de classe e/ou *de Novo* e apresentando conservação entre organismops relacionados
ncRNA novo	Inferida apenas a partir da conservação entre organismos relacionados

Além disso, se nenhuma anotação é sugerida e não há conservação entre organismos, o usuário recebe a mensagem “não anotado”.

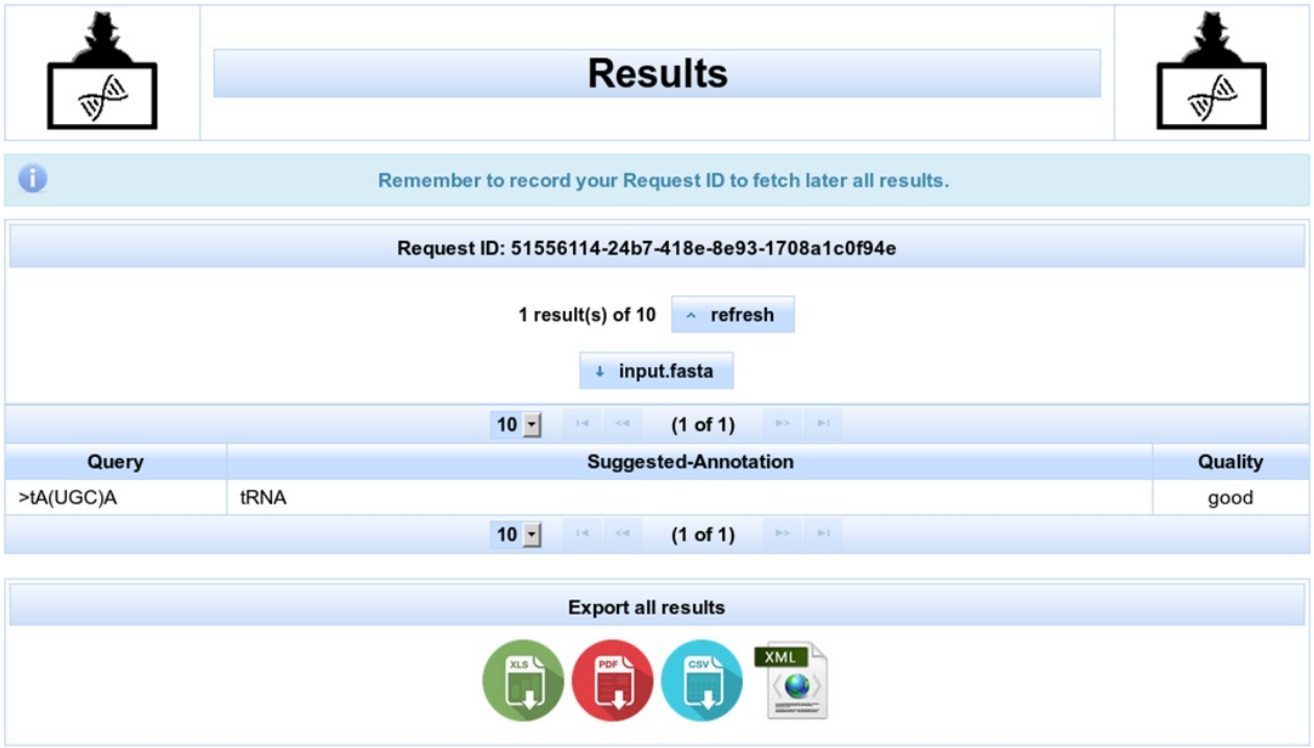


Figura 3.8: Página inicial de resultados produzidos pelo ncRNA-Agents.

Figura 3.9: Página de resultados com a anotação sugerida pelo ncRNA-Agents.

Figura 3.10: Página de resultados produzidos pela predição de classe e homologia.

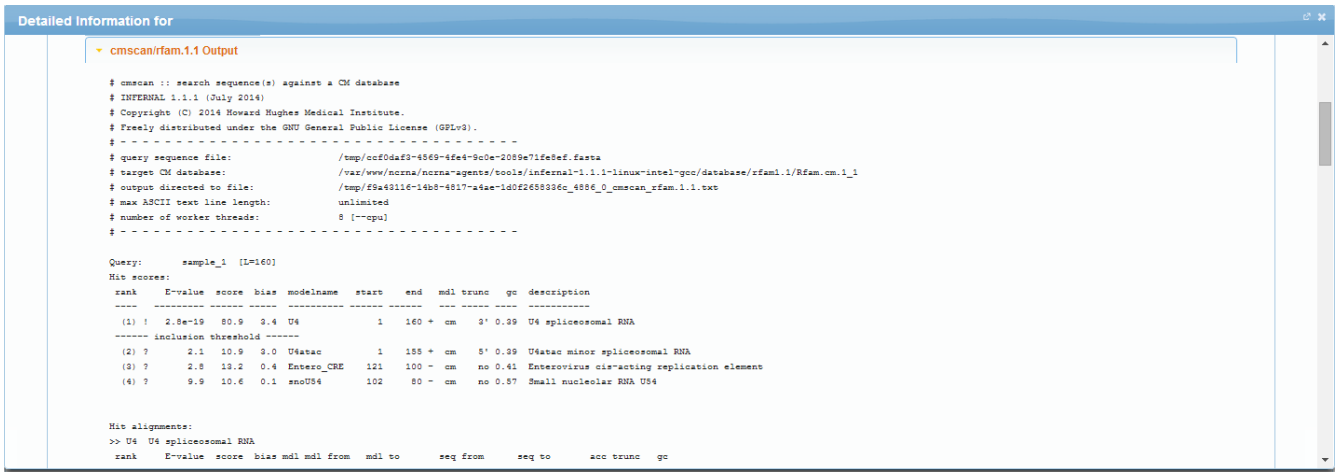


Figura 3.11: Página de resultados com análises da ferramenta Infernal.

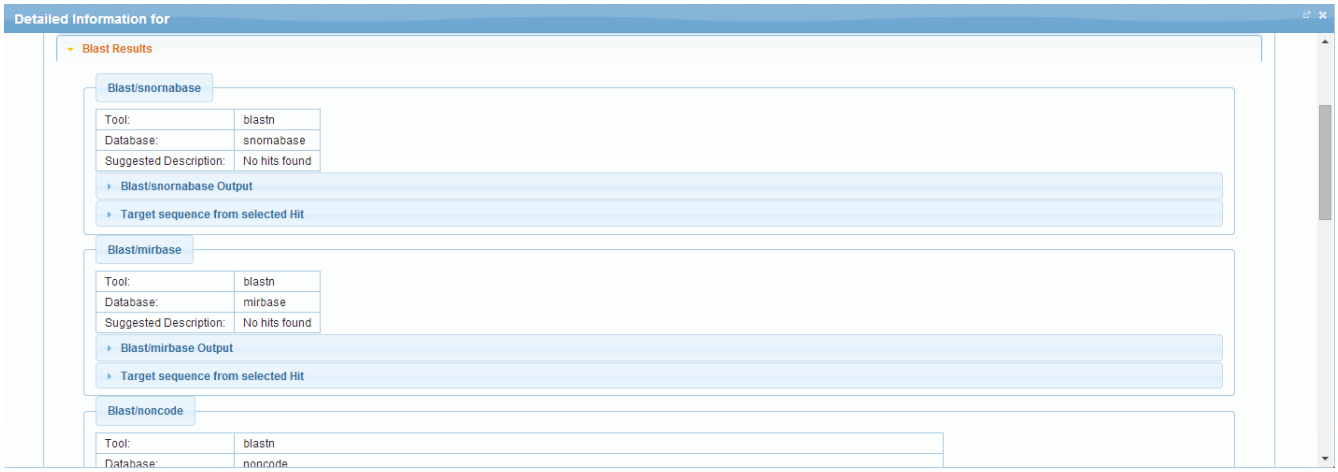


Figura 3.12: Página de resultados do BLAST.

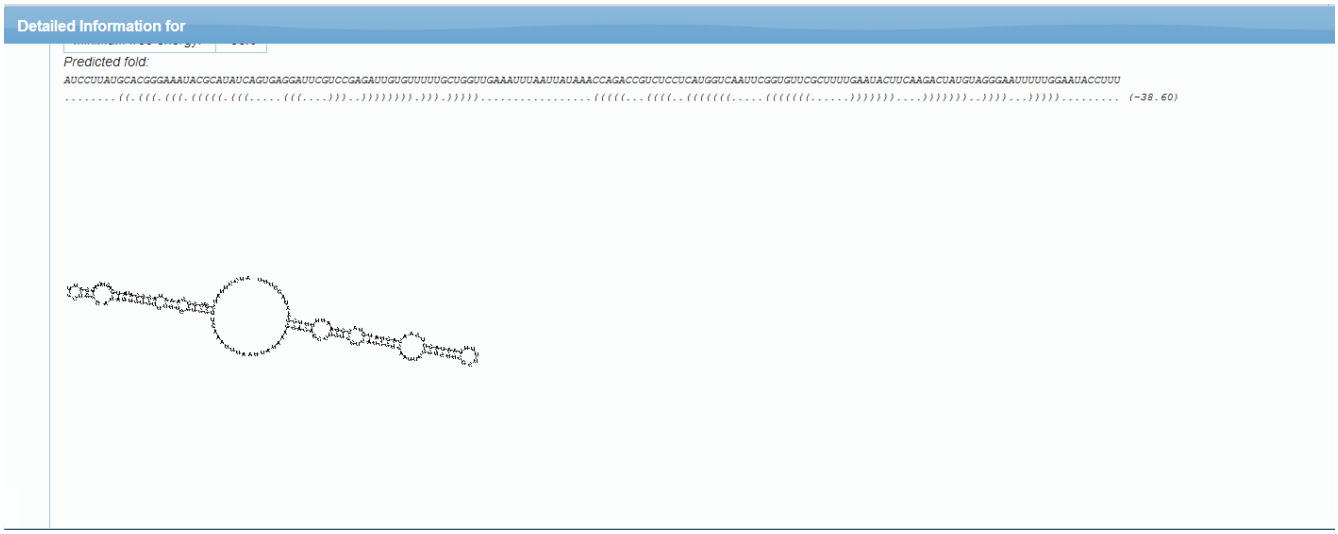


Figura 3.13: Página de resultados com a conformação espacial gerada pelo RNAfold.

Capítulo 4

Extensão do ncRNA-Agents para plantas e estudo de caso *Zea mays*

Neste capítulo serão descritas na Seção 4.1 as alterações feitas no ncRNA-Agents para a criação da nova instância do sistema. Em seguida na Seção 4.2 será apresentado o estudo de caso realizado, de encontrar ncRNAs pequenos em transcritos do milho, sendo listados seus resultados.

4.1 Uma extensão do ncRNA-Agents para plantas

Nesta seção, serão apresentadas as alterações realizadas no ncRNA-Agents para anotação de ncRNAs pequenos em transcritos de plantas (reino *Plantae*). Inicialmente, serão discutidas as alterações feitas no ncRNA-Agents. Em seguida serão detalhados os bancos de dados incluídos na nova instância do sistema para a anotação de ncRNAs de plantas.

4.1.1 Alterações no ncRNA-Agents

Foram encontradas algumas limitações no pacote RNAz já citado anteriormente, tendo em vista que, para a análise de conservação entre espécies, o RNAz é de extrema importância. Foram propostas alterações em sua execução para torná-la mais rápida.

Uma grande limitação encontrada no RNAz foi o fato de que apenas alinhamentos com no máximo seis sequências poderiam ser utilizadas. Esta restrição foi devido à quantidade limitada de conjuntos de dados disponíveis na época. Nos últimos anos, contudo, os volumes de dados cresceram massivamente e a limitação do RNAz passou a ser crítica. Para isso, utilizaremos a opção *–no–shuffle* na execução da ferramenta, para evitar o embaralhamento explícito de dados, que gera um grande atraso no processamento dos dados.

Além disso, outra limitação mostrada por estudos recentes é a de que o RNAz sofre de uma alta *false discovery rate (FDR)*. Para resolver tal problema, foi proposta a utilização de alinhamentos derivados de uma pré-filtragem com *rnazWindow.pl* com as configurações padrão (tamanho da janela 120 nt e o "tamanho do passo" (*step size*) de 40nt). Este tamanho de janela aparece suficientemente grande para detectar estruturas secundárias locais dentro de ncRNAs longos e, por outro lado, suficientemente pequeno para encontrar estruturas secundárias curtas sem perder o sinal em uma janela muito longa. A escolha

do *rnazWindow.pl* foi justificada com base em Hofacker e Stadler [26], que afirmam que, de acordo com testes feitos com alinhamentos baseados no genoma humano, utilizando-se a pré-filtragem com o *rnazWindow.pl*, evitam-se potenciais falsos positivos e aumenta-se a precisão do programa.

A proposta então é modificar a execução desse pacote no ncRNA-Agents, como descrito acima, para que fossem superadas as limitações do número de sequências comparadas e além disso houvesse o aumento da velocidade na execução do programa. Então, foi necessário adicionar ao novo sistema a entrada *rnazWindow.pl*, encontrado no *site* da TBI [57]. Esse programa por *default* segue os seguintes passos:

- Divide alinhamentos em janelas sobrepostas de tamanho 120 e deslocamento de 40.
- Verifica cada emparelhamento dos alinhamentos da sequência de referência (primeira sequência) para todas as outras sequências e, depois de remover as lacunas comuns, descarta sequências com mais de 25% de lacunas nesse alinhamento.

Assim o *rnazWindow.pl* divide os alinhamentos de entrada em janelas e faz vários filtros e pré-processamentos.

Houve a necessidade de alterar o sistema, de maneira que o *rnazWindow.pl* fosse a entrada utilizada pelo o RNAz. Assim, foi criado um *script* para iniciar automaticamente a execução do programa quando executado o RNAz.

4.1.2 Criando nova instância para plantas

Para a criação de uma nova instância do ncRNA-Agents, foi necessária a inclusão dos bancos de dados desejados (de cDNA) de plantas dos gêneros *Oryza*, *Saccharum Aegilops*, *Musa*, *Solanum*, *Triticum*. Para cada organismo, foi criado um banco *BLAST* por meio do seguinte comando, onde os nomes em letra maiúscula devem ser substituídos pelos nomes desejados:

$$makeblast - dbtypenucl - outNOME - title"TTULO - logfileNOME.log - parse\_seqids - hash\_index - inARQUIVO\_DE\_ENTRADA.fasta$$

Após a criação dos bancos para cada espécie, ainda foram necessárias algumas alterações no ncRNA-Agents para o acesso correto aos novos bancos de dados, para referenciá-los no arquivo de configuração e para adicioná-los na lista de bancos de conservação da interface.

As sequências de cDNA dos gêneros já citados nesse trabalho foram obtidos do *site* http://plants.ensembl.org/species.html, compreendendo os seguintes organismos:

- *Oryza barthii*;
- *Oryza brachyantha*;
- *Oryza nivara*;
- *Oryza punctata*;
- *Oryza rufipogon*;

- *Saccharum officinarum*;
- *Aegilops tauschii*;
- *Solanum tuberosum*;
- *Triticum aestivum*;
- *Triticum urartu*;
- *Shorgum bicolor*.

4.1.3 Ferramentas utilizadas para realização do estudo de caso

As classes *Homologia, Predição de Classe, De Novo e Conservação* foram utilizadas para anotar os resultados gerados e as ferramentas utilizadas foram:

- Blast com os bancos: snoRNABase, RNAdb, NONCODE, miRBase e Plant-snoRNA;
- Infernal;
- tRNAscan;
- SVM-Portrait;
- snoReport;
- RNAfold;
- Blast com os bancos das plantas: *Oryza barthii*, *Oryza brachyantha*, *Oryza nivara*, *Oryza punctata*, *Oryza rufipogon*, *Saccharum officinarum*, *Aegilops tauschii*, *Solanum tuberosum*, *Triticum aestivum*, *Triticum urartu*, *Shorgum bicolor*.

4.2 Estudo de caso: *Zea mays*

Nesta seção, será discutido o estudo de caso com o *Zea mays* (milho) realizado executando-se o ncRNA-Agents instanciado para plantas. Inicialmente, será descrito o objeto do estudo de caso, o milho. Em seguida serão discutidos os experimentos feitos para o estudo de caso e serão descritos os dados utilizados para a realização dos experimentos. Finalmente serão listados os resultados das anotações de de ncRNAs pequenos do milho.

4.2.1 Sobre o *Z. mays*

O milho é um cereal pertencente à família das *Poaceae*, da subfamília: Panicoidea, do gênero: *Zea* e da espécie: *Zea mays L.* e o seu ancestral conhecido como teosinto: *Zea mays* mexicana [55].

O milho pertence ao grupo das angiospermas, que produzem as sementes no fruto. É uma planta sub-arbustiva de colmo nodoso. É a variante domesticada do teosinto, as duas plantas possuem aparência semelhante, milho com um pedúnculo[1] único, alto com

[1]Haste que sustenta uma inflorescência, ou a flor de uma inflorescência simples, e posteriormente o(s) fruto(s).

folhas múltiplas e o teosinto sendo uma curta, frondosa planta. A diferença entre os dois é controlada por diferenças em apenas dois genes [55].

O milho é um dos cerais mais importantes cultivado neste continente, isso se deve a sua capacidade de ser utilizado como alimento e na produção animal. A partir do milho também pode ser obtido o etanol, utilizado atualmente como combustível. Todas as evidências científicas levam a crer que seja uma planta de origem mexicana, já que a sua domesticação começou 7.500 a 12.000 anos atrás na área central da Mesoamérica. Contém a maioria dos os aminoácidos conhecidos, exceto a lisina e o triptofano [55].

Tem um alto potencial produtivo e é bastante responsivo à tecnologia. Seu cultivo geralmente é mecanizado, beneficiando-se muito de técnicas modernas de plantio e colheita.

Uma das variedades mais difundidas no Brasil é o milho branco que tem como principais finalidades a produção de canjica, grãos e silagem. A planta tem altura próxima de 2,20 metros, sendo que a espiga nasce a 1,10 metros do solo. A espiga é grande, cilíndrica e apresenta alta compensação. O sabugo é fino, os grãos são brancos, profundos, pesados e de textura média. O colmo tem alta resistência física e boa sanidade. A raiz tem boa fixação. A planta é especialmente resistente às principais doenças foliares do milho, em diferentes altitudes e épocas de plantio (Figura 4.1).

Figura 4.1: A planta *Zea mays*, popularmente conhecida como milho [15].

4.2.2 Dados do experimento

Em seguida uma descrição mais detalhada dos dados utilizados na realização do estudo de caso feito para o milho.

Inoculação do Milho com bactérias diazotróficas

O nitrogênio é considerado de grande importância no cultivo das plantas pois atua no metabolismo das mesmas, principalmente na síntese proteica. Assim, o nitrogênio é fundamental quando se fala na elevação da produção de grãos e do seu nível proteico. Contudo os fertilizantes nitrogenados são considerados como fonte de risco de poluição ambiental dos sistemas agrícolas [55].

A fixação biológica feita por bactérias diazotróficas se mostra como alternativa ao uso de fertilizantes nitrogenados. O uso dessas bactérias tem provado trazer alguns benefícios, entre eles, o crescimento vegetal, principalmente das raízes, e maximização da absorção de nutrientes e da água.

Essas bactérias, também chamadas de bactérias promotoras de crescimento de plantas (BPCP), pode estimular o crescimento das plantas por diversas maneiras, sendo as mais importantes:

- Capacidade de fixação biológica de nitrogênio [27];
- Aumento na atividade na redutase do nitrato quando crescem endofiticamente nas plantas [10];
- Produção de hormônios como auxinas, citocininas [58], giberilinas [6], etileno [56], e uma variedade de outras moléculas;
- Solubilização do fosfato [46];
- Atuam com agente de controle biológico de patógenos [12].

Os dados analisados neste trabalho para testar o ncRNA-Agents instanciado para plantas foram disponibilizados pelo prof. Paulo Cavalcanti, da Universidade Federal do Rio de Janeiro [21]. Os dados são de RNAs da *Zea mays* inoculados, ou não, pelas bactérias *Azospirillum* e *Herbaspirillum*.

O grupo *Azospirillum* abrange um grupo de BPCP de vida livre que é encontrado na maior parte da terra. Existem relatos de que as bactérias desse gênero podem ser endofíticas facultativas[2]. A espécie *Spirillum lipoferum* foi inicialmente descrita por Beijerinck [28] e, em 1978, foi proposta a sua reclassificação com *Azospirillum*, juntamente com a criação de duas espécies:*Azospirillum lipoferum* e *Azospirillum brasilense*.

Bactérias do gênero *Azospirillum* ganharam grande destaque mundial a partir da década de 1970 com a descoberta pela pesquisadora da Embrapa, Dra. Johanna Dobereiner [28], da capacidade de fixação biológica do nitrogênio dessas bactérias quando em associação com gramíneas. A propriedade de fixar nitrogênio em vida livre foi responsável pela mudança no nome do gênero *Spirillum*, sendo adicionado o prefixo "azo", alusivo ao nome utilizado por Lavoisier para denominar o elemento nitrogênio [28].

Herbaspirillum é um gênero de bactérias, incluindo a fixação de nitrogênio feito pela espécie *Herbaspirillum lusitanum*, sendo esta também encontrada em nódulos radiculares de feijão (*Phaseolus vulgaris*).

Dados utilizados no experimento

Como já foi dito, o trabalho com milho e seu tratamento com *Azospirillum* e *Herbaspirillum* está em desenvolvimento na Universidade Federal do Rio de Janeiro (UFRJ) [21]. A partir do milho sequenciado foram obtidos dados em oito bibliotecas:

[2]Bactérias diazotróficas endofíticas são aquelas que fixam N_2 atmosférico e colonizam o interior de tecidos vegetais sem causar sintomas de doenças. Contribuem para o desenvolvimento das plantas por meio da fixação biológica do nitrogênio, produção e liberação de substâncias reguladoras do crescimento vegetal, podendo, assim, facilitar a revegetação de solos degradados por atividades antrópicas [38].

- As de ids 9 e 11 funcionam como controle;
- As de ids 10 e 12 são as bibliotecas tratadas com Herbaspirillum;
- As de ids 29 e 30 são as bibliotecas tratadas com Azospirillum;
- As de ids 31 e 32 funcionam como controle.

Para obter os oito arquivos de entrada para o ncRNA-Agents os dados do sequenciamento feito pelo prof. Cavalcanti passaram pelas etapas mostradas na Figura 4.2.

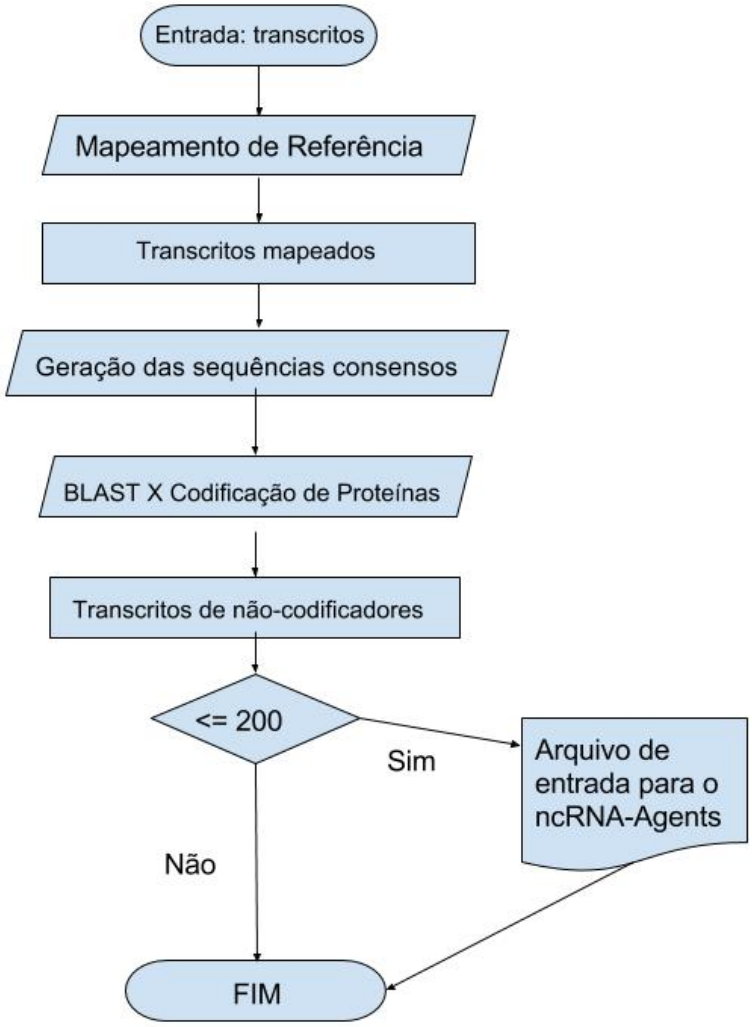


Figura 4.2: Fluxograma que mostra como são gerados os arquivos de entrada para o ncRNA-Agents. Os oito arquivos de entrada com os transcritos, foram submetidos a uma fase de mapeamento, com um organismo de referência (milho). Para remover os consensos que são anotados como proteínas, foi realizado um BLAST, utilizando-se um banco de dados com transcritos do milho. Por último, apenas os transcritos com comprimento menor ou igual a 200 *pb* (uma característica-chave na previsão de pequenos ncRNAs) são selecionados.

Assim, foram submetidos ao ncRNA-Agents arquivos de sequências no formato *fasta* (Figura 4.3) contendo todos os RNAs da planta *Z. mays* inoculados pelas bactérias *Azospirillum* e *Herbaspirillum*, além dos arquivos com os RNAs das bibliotecas de controle.

```
>transcript:Zm00001d027252_T001 gene=gene:Zm00001d027252
GTCAGGATAGCTCAGTTGGTAGAGCAGAGGACTGAAAATCCTCGTGTCACCAGTTCAAATCTGGTTCCTGGCA
>transcript:Zm00001d022651_T001 gene=gene:Zm00001d022651
CCTACATCACCTCCCATTCCGCCTTTGGCCCCGCAACCGGCGTCCCTCTCCGTTGATGGACTCCTACTCCTCCCCCTCGGCGGTACCGCCGG
>transcript:Zm00001d027316_T001 gene=gene:Zm00001d027316
GCTGGAATAGCTCAGTTGGTTAGAGCGTGTGGCTGTTAACCACAAGGTCGGAGGTTCAAGCCCTCCTTCCAGCG
>transcript:Zm00001d027327_T001 gene=gene:Zm00001d027327
GCCCCTATAGCTCAGTGGTAGAGCGTCAGTCTTGTAAACTGAAGGTCCGTAGTTCGATCCTGCGTGGGGGCA
>transcript:Zm00001d027378_T001 gene=gene:Zm00001d027378
GCCTCTAGCCTAGTGGTTAAGGCTTCTGAGTAGCACCTCCAAGTCCCAGGTTCGATTCCCCTCGGGGGCG
>transcript:Zm00001d027381_T001 gene=gene:Zm00001d027381
gtccatctagcccacttggtagagcacaaggcttctaaccatgtggtcgtgggttcaagccccatagtttgca
>transcript:Zm00001d027382_T001 gene=gene:Zm00001d027382
gcccatctagcccacttggtagagcacaaggcttctaaccatgtggttgtgggttcaagccccatagtttgca
>transcript:Zm00001d027396_T001 gene=gene:Zm00001d027396
acttgtctagctcagttggtagagctcaaggcttttaaccttgtggttgtgggttcaagccccatgataggta
>transcript:Zm00001d027397_T001 gene=gene:Zm00001d027397
gtatgtctagctcagtcggtagagtgcaagacttttaactttgaggttgtgggttcaagctccatcgtggacg
>transcript:Zm00001d027465_T001 gene=gene:Zm00001d027465
GCTGGAATAGCTCAGTTGGTTAGAGCGTATGGCTGTTAACCATAAGGTCGGAGGTTCAAGCCCTCCTTCTAGCG
```

Figura 4.3: Exemplo de arquivo no formato *fasta*. O *fasta* é um dos formatos mais utilizados na área de Bioinformática, tendo na primeira linha um identificador de sequência após o caractere '>', e nas próximas linhas a sequência do genoma/transcrito.

As Tabelas 4.1 e 4.2 mostram resultados para duas das bibliotecas utilizadas, uma do milho tratado com *Azospirillum* e outra com o milho tratado com a *Herbaspirillum*.

Tabela 4.1: Dados do número de sequências processadas para criar os arquivos de entrada para os experimentos.

Azospirillum - biblioteca 29	
Entrada Bruta	5.572.730
Entrada filtrada (somente consensos)	161.477
Arquivo de entrada após filtragem	2.334

Tabela 4.2: Dados do número de sequências processadas para criar os arquivos de entrada para os experimentos.

Herbaspirillum - biblioteca 12	
Entrada Bruta	5.567.480
Entrada filtrada (somente consensos)	155.578
Arquivo de entrada após filtragem	2.262

4.2.3 Resultados

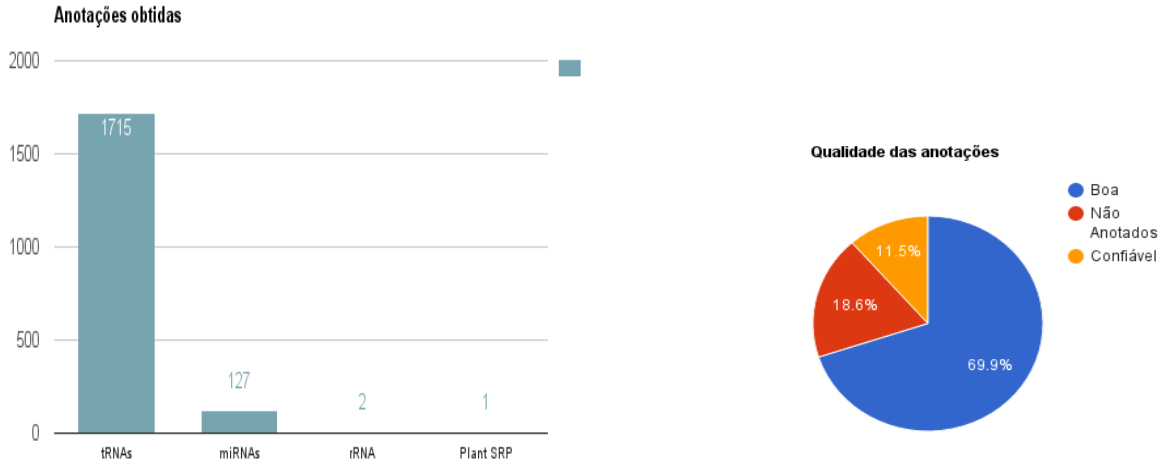
Foram submetidos oito arquivos de entrada, conforme descrito anteriormente, dois destes arquivos continham dados de pequenos ncRNAs do milho tratados com a bactéria *Azospirillum*, mais dois tratados com a bactéria *Herbaspirillum* e quatro que funcionavam como controle.

Os resultados encontrados serão apenas relatados neste trabalho pois estes necessitam de uma análise mais aprofundada que deverá ser feita pelo prof. Paulo Cavalcanti. Sendo assim, a seguir se encontram os resultados obtidos para os oito arquivos submetidos ao ncRNA-Agents.

Tabela 4.3: Resultados para biblioteca 9 - Controle.

Total de sequências	2.266
Sequências anotadas	1.846
Sequências não anotadas	420

Para a biblioteca 09, a Tabela 4.3 mostra um total de 2.266 sequências de entrada na qual 420 não foram anotadas e 1.846 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 127 anotações de miRNA, 2 anotações de rRNA e uma anotação de plant SRP[3] como mostra a Figura 4.4(a).



(a) (b)

Figura 4.4: Mais resultados para a biblioteca 09, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

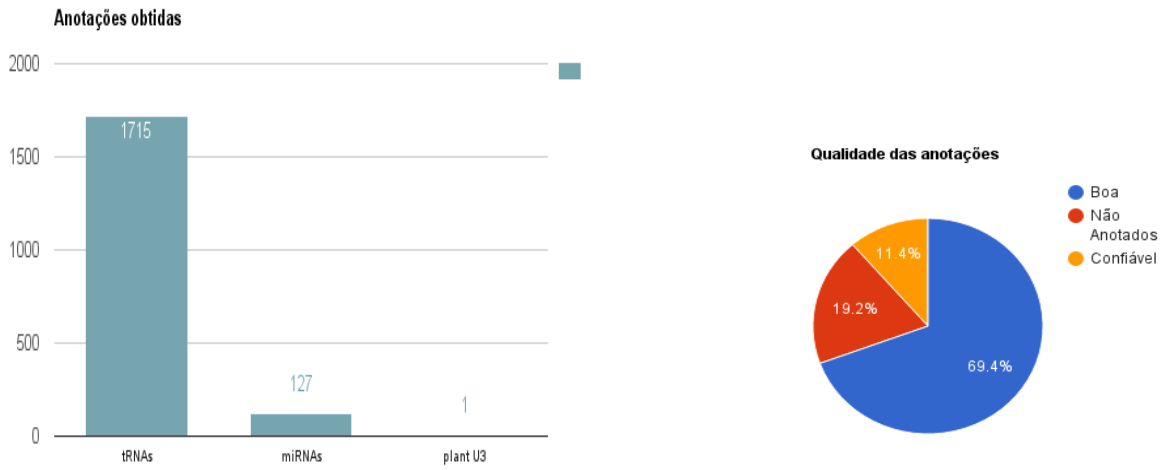
Tabela 4.4: Resultados para biblioteca 11 - Controle

Total de sequências	2.286
Sequências anotadas	1.846
Sequências não anotadas	440

Para a biblioteca 11, a Tabela 4.4 mostra um total de 2.286 sequências de entrada na qual 440 não foram anotadas e 1.846 tiveram diferentes anotações. Das que foram

[3]Plant signal recognition particle RNA - são partículas de reconhecimento de sinal que tem como componente uma partícula de RNA. Tipicamente, SRP-RNAs consistem em cerca de 300 nucleótidos e são contactados diretamente por proteínas [34].

anotadas, obteve-se 1.715 anotações de tRNA, 127 de miRNA e 1 de plant U3 como mostra a Figura 4.5(a).



(a) (b)

Figura 4.5: Mais resultados para a biblioteca 11, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

Tabela 4.5: Resultados para biblioteca 10 - *Herbaspirillum.*

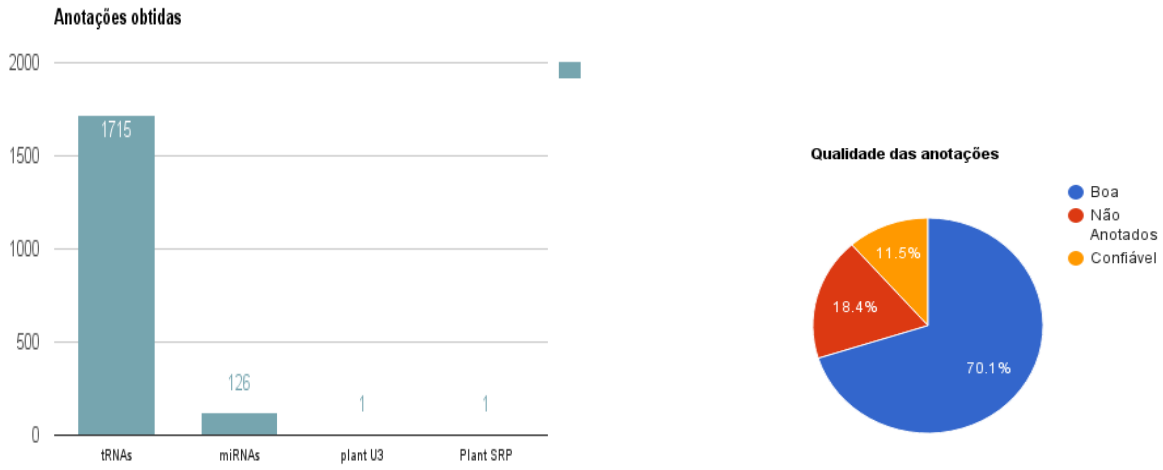
Total de sequências	2.260
Sequências anotadas	1.844
Sequências não anotadas	416

Para a biblioteca 10, a Tabela 4.5 mostra um total de 2.260 sequências de entrada na qual 416 não foram anotadas e 1.844 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 126 de miRNA, 1 de plant U3[4] e uma de plant SRP como mostra a Figura 4.6(a).

Tabela 4.6: Resultados para biblioteca 12 - *Herbaspirillum.*

Total de sequências	2.262
Sequências anotadas	1.844
Sequências não anotadas	418

[4]Small nucleolar RNA U3 (snoRNA U3).



(a) (b)

Figura 4.6: Mais resultados para a biblioteca 10, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

Para a biblioteca 12, a Tabela 4.6 mostra um total de 2.262 sequências de entrada na qual 418 não foram anotadas e 1.844 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 126 anotações de miRNA, 2 anotações de rRNA e 1 anotação de plant U3 como mostra a Figura 4.7(a).

Tabela 4.7: Resultados para biblioteca 29 - *Azospirillum*.

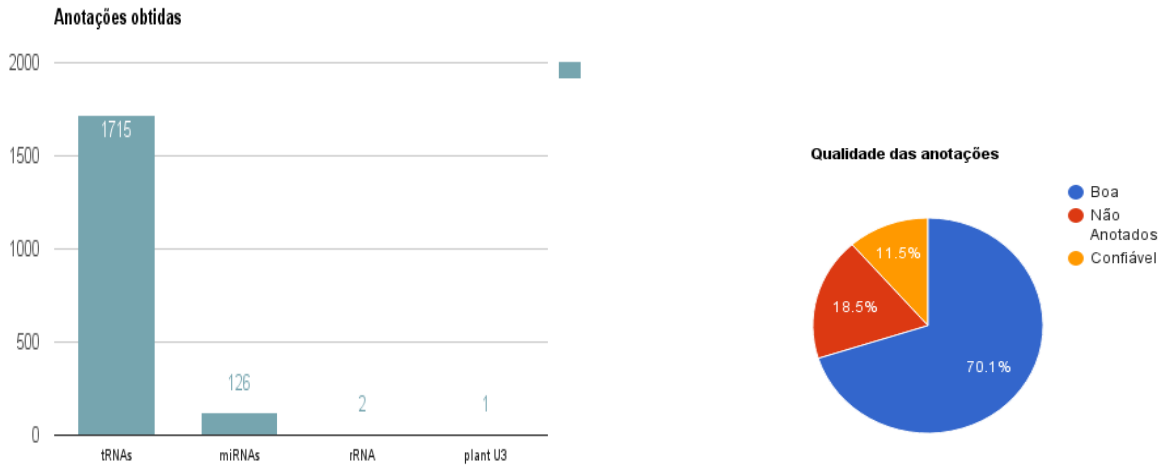
Total de sequências	2.234
Sequências anotadas	1.746
Sequências não anotadas	488

Para as bibliotecas 9, 11, 10 e 12 foram anotadas 7.380 sequências. O diagrama apresentado na Figura 4.8 faz relação entra as anotações das bibliotecas de controle e bibliotecas inoculadas com *Herbaspirillum*.

Para a biblioteca 29, a Tabela 4.7 mostra um total de 2.234 sequências de entrada na qual 488 não foram anotadas e 1.746 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 126 de miRNA, 1 de rRNA, 1 de Plant SRP e 1 de U2[5] como mostra a Figura 4.9(a).

Para a biblioteca 30, a Tabela 4.8 mostra um total de 2.316 sequências de entrada na qual 472 não foram anotadas e 1.844 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 126 anotações de miRNA e 1 anotação de U2 como mostra a Figura 4.10(a).

[5]U2 spliceosomal RNA - snRNA U2



(a) (b)

Figura 4.7: Mais resultados para a biblioteca 12, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

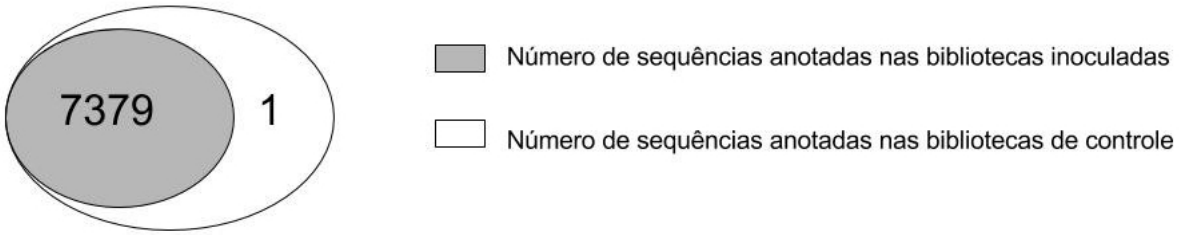


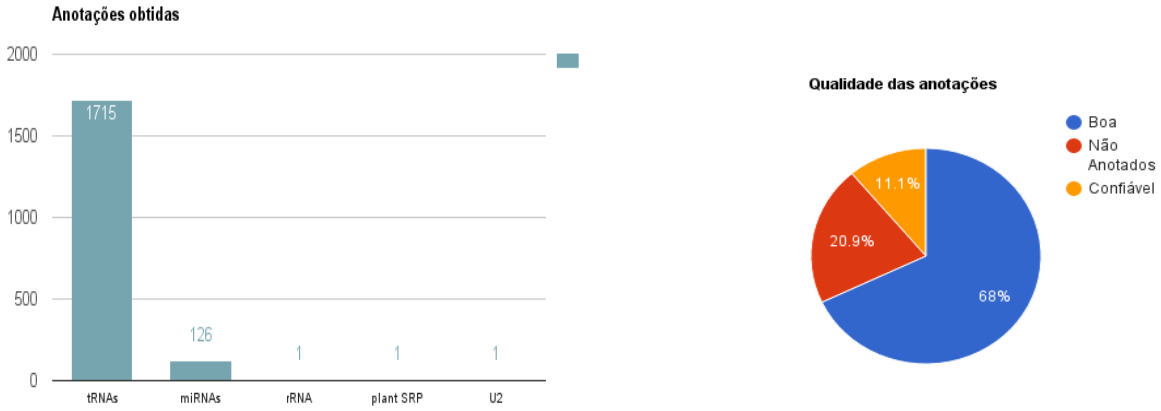
Figura 4.8: Diagrama de Venn que relaciona as anotações das bibliotecas de controle e bibliotecas inoculadas com *Herbaspirillum*.

Tabela 4.8: Resultados para biblioteca 30 - *Azospirillum*.

Total de sequências	2.316
Sequências anotadas	1.844
Sequências não anotadas	472

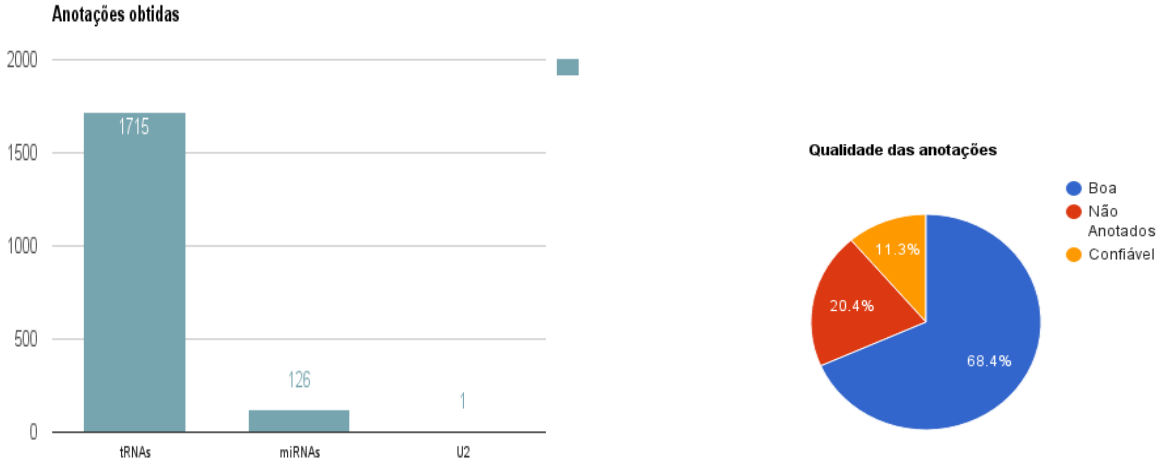
Para a biblioteca 31, a Tabela 4.9 mostra um total de 2.374 sequências de entrada na qual 527 não foram anotadas e 1.847 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 127 anotações de miRNA, 1 anotação de snoRNA (snoR30), 1 anotação de rRNA e 2 anotações de Plant SRP como mostra a Figura 4.11(a).

Para a biblioteca 32, a Tabela 4.10 mostra um total de 2.341 sequências de entrada



(a) (b)

Figura 4.9: Mais resultados para a biblioteca 29, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotada.



(a) (b)

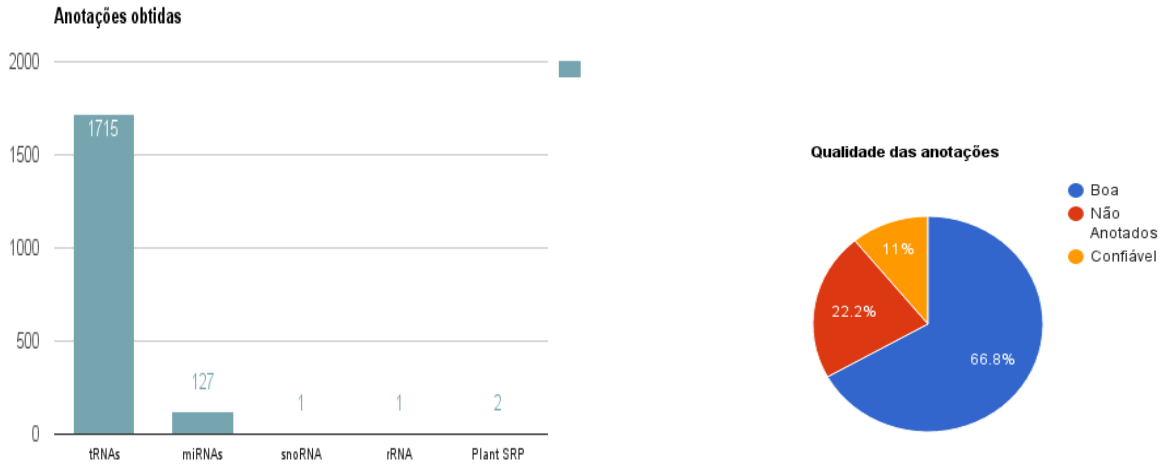
Figura 4.10: Mais resultados para a biblioteca 30, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

na qual 488 não foram anotadas e 1.853 tiveram diferentes anotações. Das que foram anotadas, obteve-se 1.715 anotações de tRNA, 127 anotações de miRNA, 6 anotações de rRNA e 1 anotação de Plant SRP como mostra a Figura 4.12(a).

Para as bibliotecas 29, 30, 31 e 32 foram anotadas 7.290 sequências. O diagrama apresentado na Figura 4.13 faz relação entra as anotações das bibliotecas de controle e bibliotecas inoculadas com *Azospirillum*.

Tabela 4.9: Resultados para biblioteca 31 - Controle.

Total de sequências	2.374
Sequências anotadas	1.847
Sequências não anotadas	527



(a) (b)

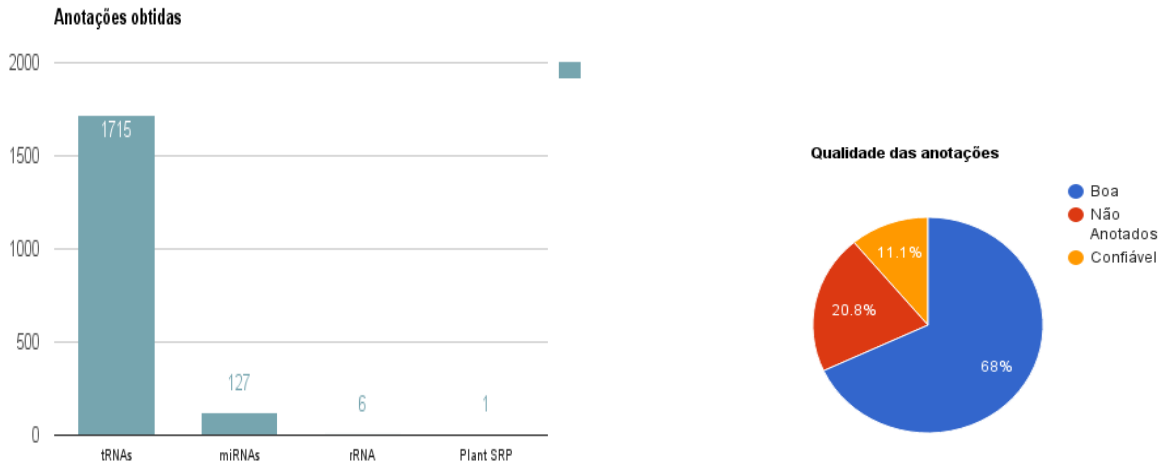
Figura 4.11: Mais resultados para a biblioteca 31, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

Tabela 4.10: Resultados para biblioteca 32 - Controle.

Total de sequências	2.341
Sequências anotadas	1.853
Sequências não anotadas	488

É possível comparar as anotações feitas para as bibliotecas 9, 10, 11 e 12 que são relacionadas com a bactéria *Herbaspirillum* com as anotações das bibliotecas 29, 30, 31 e 32 que são relacionadas com a bactéria *Azospirillum*. Essa comparação é mostrada no diagrama apresentado na Figura 4.14. É interessante notar que grande parte dos ncRNAs pequenos são comuns entre as bibliotecas, mas aparecem ncRNAs exclusivos de Azospirillum (U2 snoRNA) e em Herbaspirillum (Plant U3), notando-se que ambos são snoRNAs.

No Anexo I está a lista completa de todas as anotações executadas.



(a) (b)

Figura 4.12: Mais resultados para a biblioteca 32, respectivamente, (a) anotações de ncRNAs; (b) qualidade das sequências anotadas.

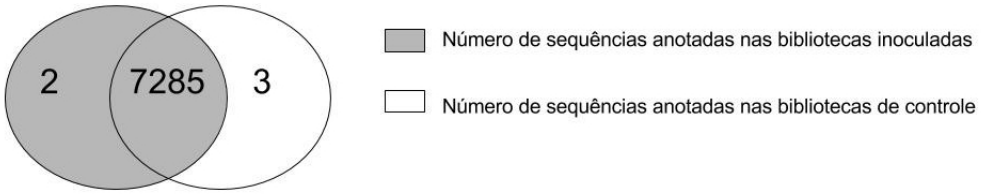


Figura 4.13: Diagrama de Venn que relaciona as anotações das bibliotecas de controle e bibliotecas inoculadas com *Azospirillum*.

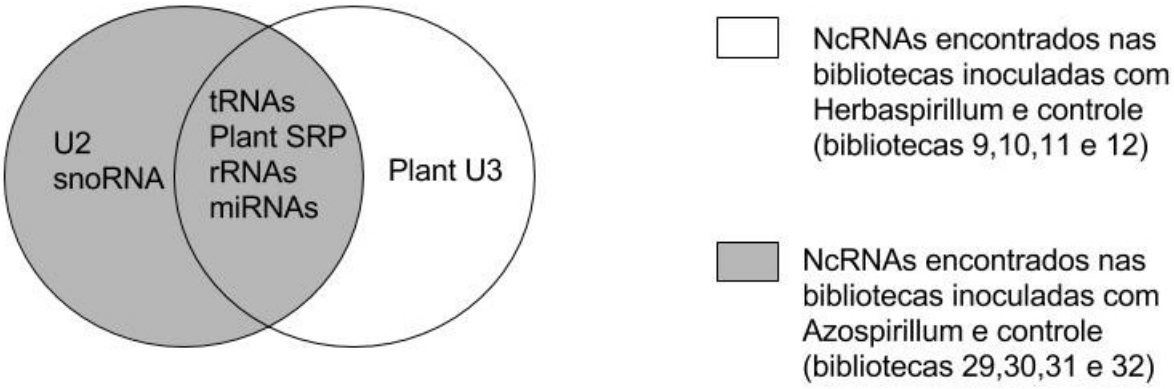


Figura 4.14: Comparação das anotações das bibliotecas de controle e inoculadas com *Azospirillum* com as anotações das bibliotecas de controle e inoculadas com *Herbaspirillum*.

Capítulo 5

Conclusões e Trabalhos Futuros

O ncRNA-Agents é um sistema de anotação de ncRNAs baseado no conceito de Sistemas Multiagentes (SMAs), primeiramente foi desenvolvida uma instância do sistema para anotação de fungos, então para este trabalho, foi criada de uma nova instância do ncRNA-Agents, permitindo que o usuário pudesse anotar ncRNAs de plantas. Essa nova instância do ncRNA-Agents, contém novos bancos de dados de plantas das famílias *Gramíneas* e *Solanaceae*, o que proporciona melhor análise de conservação entre plantas para o milho. Foi desenvolvido um estudo de caso para a *Zea mays* com quatro bibliotecas geradas por inoculação pelas bactérias *Azospirillum* e *Herbaspirillum* e mais quatro bibliotecas de controle. Essas oito bibliotecas foram filtradas, para a remoção das sequências que foram anotadas como proteínas e ainda remoção daquelas que tivessem tamanho maior que 200 bp. As sequências com menos de 200 *bp*, ou seja, as sequências com candidatos a ncRNAs pequenos, foram submetidas ao ncRNA-Agents, para anotação. Das oito bibliotecas foram submetidas aproximadamente 18.300 sequências, e foram encontrados diferentes tipos de ncRNAs, sendo em sua maioria RNAs transportadores (tRNAs) e *micro* RNAs (miRNAs), sendo encontrados também RNAs ribossomais (rRNA), *Small nucleolar* RNAs U3 (snoRNA U3), *Plant signal recognition particle* RNA (Plant SRP) e U2 *spliceosomal* RNA (snRNA U2). Os resultados obtidos foram listados e se encontram completos no Anexo I, estes serão enviados para o prof. Cavalcanti para análise.

5.1 Contribuições

As principais contribuições deste trabalho foram:

- NcRNA-Agents instanciado para plantas, tendo sido incluídos bancos de dados de diversos gêneros do reino *Plantae*;
- Estudo de caso para o *Z. mays* (milho), onde foram executados no ncRNA-Agents oito arquivos *fasta* contendo transcritos de RNAs do milho, inoculado pelas bactérias *Azospirillum* e *Herbaspirillum*, além das bibliotecas de controle. Os resultados obtidos foram relatados.

5.2 Trabalhos futuros

As perspectivas para este trabalho são:

- Disponibilizar o ncRNA-Agents *Plantae* com os novos bancos de dados incluídos para o prof. Paulo Cavalcanti da UFRJ;
- Criar novas instâncias do ncRNA-Agents para outros reinos.

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Anexo I

Lista de anotações

Tabela com os resultados das bibliotecas utilizadas no experimento do prof. Paulo Cavalcanti e executada no ncRNA-Agents instanciado descrito neste trabalho.

Query	Suggested-Annotation	Quality
>CUFF.5590.1	MIR8576 MI0027461 Amborella tricho-poda miR8576 stem-loop	good
>transcript:zma-mir1432	MIR1432 MI0013243 Zea mays miR1432 stem-looplcl|n361524	good
>transcript:zma-mir166h	MIR166h MI0001482 Zea mays miR166h stem-looplcl|n351633	good
>transcript:zma-mir169l	MIR169l MI0013199 Zea mays miR169l stem-looplcl|n361480	good
>transcript:zma-mir169m	MIR169m MI0013200 Zea mays miR169m stem-looplcl|n361481	good
>transcript:zma-mir169n	MIR169n MI0013201 Zea mays miR169n stem-looplcl|n361482	good
>transcript:zma-mir169o	MIR169o MI0013202 Zea mays miR169o stem-looplcl|n361483 |	good
>transcript:zma-mir169q	MIR169q MI0013204 Zea mays miR169q stem-looplcl|n361486	good
>transcript:zma-mir2275a	MIR2275a MI0011278 Zea mays miR2275a stem-looplcl|n359632	good
>transcript:zma-mir2275b	MIR2275b MI0011279 Zea mays miR2275b stem-looplcl|n359633	good
>transcript:zma-mir390a	MIR390a MI0013209 Zea mays miR390a stem-looplcl|n361490	good
>transcript:zma-mir393a	MIR393a MI0001845 Zea mays miR393a stem-looplcl|n351929	good
>transcript:zma-mir393b	MIR393b MI0013210 Zea mays miR393b stem-looplcl|n361496	good
>transcript:zma-mir393c	MIR393c MI0013211 Zea mays miR393c stem-looplcl|n361494	good

>transcript:zma-mir395f	MIR395f MI0013214 Zea mays miR395f stem-looplcl|n361499	good
>transcript:zma-mir396c	MIR396c MI0010569 Zea mays miR396c stem-looplcl|n358952	good
>transcript:zma-mir396d	MIR396d MI0010570 Zea mays miR396d stem-looplcl|n358953	good
>transcript:zma-mir398b	MIR398b MI0013232 Zea mays miR398b stem-looplcl|n361515	good
>transcript:zma-mir399g	MIR399g MI0013233 Zea mays miR399g stem-looplcl|n361513	good
>transcript:zma-mir399h	MIR399h MI0013234 Zea mays miR399h stem-looplcl|n361514	good
>transcript:zma-mir399i	MIR399i MI0013235 Zea mays miR399i stem-looplcl|n361522	good
>transcript:zma-mir399j	MIR399j MI0013236 Zea mays miR399j stem-looplcl|n361523	good
>transcript:zma-mir528a	MIR528a MI0013239 Zea mays miR528a stem-looplcl|n361525	good
>transcript:zma-mir528b	MIR528b MI0013240 Zea mays miR528b stem-looplcl|n361518	good
>transcript:zma-mir156h	mir-156	good
>transcript:zma-mir156e	mir-156	good
>transcript:zma-mir156b	mir-156	good
>transcript:zma-mir156a	mir-156	good
>transcript:zma-mir156i	mir-156	good
>transcript:zma-mir156l	mir-156	good
>transcript:zma-mir156k	mir-156	good
>transcript:zma-mir156j	mir-156	good
>transcript:zma-mir156d	mir-156	reliable
>transcript:zma-mir160f	mir-160	good
>transcript:zma-mir160c	mir-160	good
>transcript:zma-mir160d	mir-160	reliable
>transcript:zma-mir160b	mir-160	reliable
>transcript:zma-mir160g	mir-160	reliable
>transcript:zma-mir166i	mir-166	good
>transcript:zma-mir166l	mir-166	good
>transcript:zma-mir166d	mir-166	good
>transcript:zma-mir166k	mir-166	good
>transcript:zma-mir166m	mir-166	good
>transcript:zma-mir166j	mir-166	good
>transcript:zma-mir166c	mir-166	reliable
>transcript:zma-mir172e	mir-172	good
>transcript:zma-mir172c	mir-172	good
>transcript:zma-mir172b	mir-172	good

>transcript:zma-mir172d	mir-172	good
>transcript:zma-mir395k	mir-395	good
>transcript:zma-mir395l	mir-395	good
>transcript:zma-mir395m	mir-395	good
>transcript:zma-mir395c	mir-395	good
>transcript:zma-mir395n	mir-395	good
>transcript:zma-mir395p	mir-395	good
>transcript:zma-mir395d	mir-395	good
>transcript:zma-mir395e	mir-395	good
>transcript:zma-mir395i	mir-395	good
>transcript:zma-mir395b	mir-395	good
>transcript:zma-mir395a	mir-395	good
>transcript:zma-mir399d	mir-399	good
>transcript:zma-mir399b	mir-399	good
>transcript:zma-mir399f	mir-399	good
>transcript:zma-mir399e	mir-399	reliable
>transcript:zma-mir399a	mir-399	reliable
>transcript:zma-mir162	MIR162_2	good
>transcript:zma-mir164e	MIR164	good
>transcript:zma-mir164a	MIR164	good
>transcript:zma-mir164f	MIR164	good
>transcript:zma-mir164g	MIR164	good
>transcript:zma-mir164b	MIR164	good
>transcript:zma-mir167j	MIR167_1	good
>transcript:zma-mir167a	MIR167_1	good
>transcript:zma-mir167c	MIR167_1	good
>transcript:zma-mir167b	MIR167_1	good
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Tabela I.4: Tabela da biblioteca 12

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Tabela I.6: Tabela da biblioteca 30

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